The Fundamentals Of Tissue Arrays

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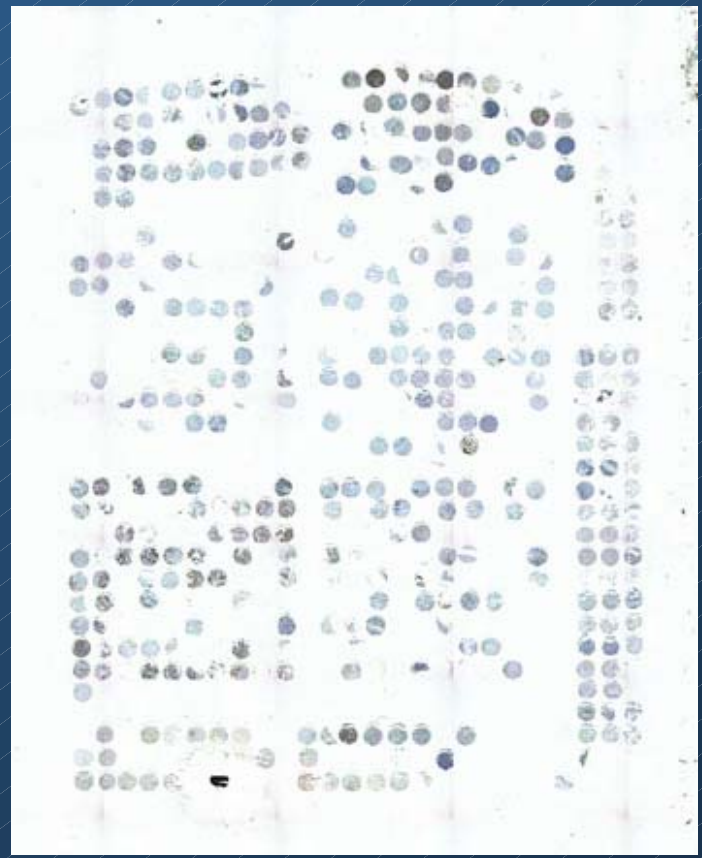
What Is A Tissue Array?

- *A Block Of Samples From Hundreds Of Blocks*

- Multiple Samples
- Paraffin Embedded Tissue
- Arranged In An Organized Fashion

What Can A Tissue Array Be Used For?

- Immunohistochemistry
- Immunofluoresence
- In Situ Hybridization
- FISH
- Histochemical Stains



Why Tissue Arrays ?

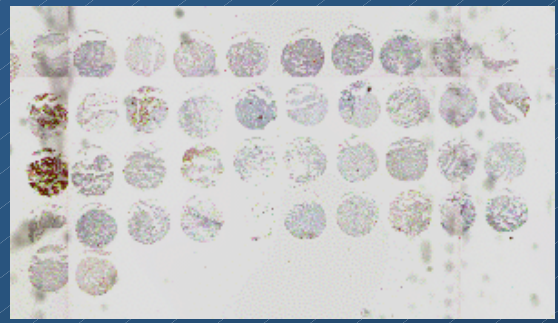
- New Antibodies For Diagnostics
- New Prognostic Markers
- Target Verification For Micro-Arrays

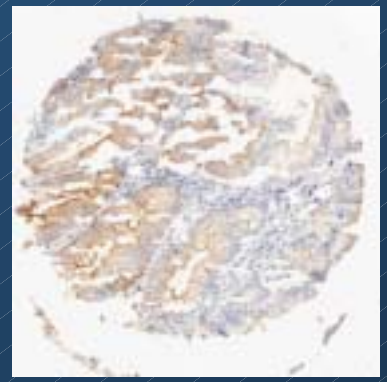
- Time Courses
- Developmental Biology
- Treated Vs. Untreated

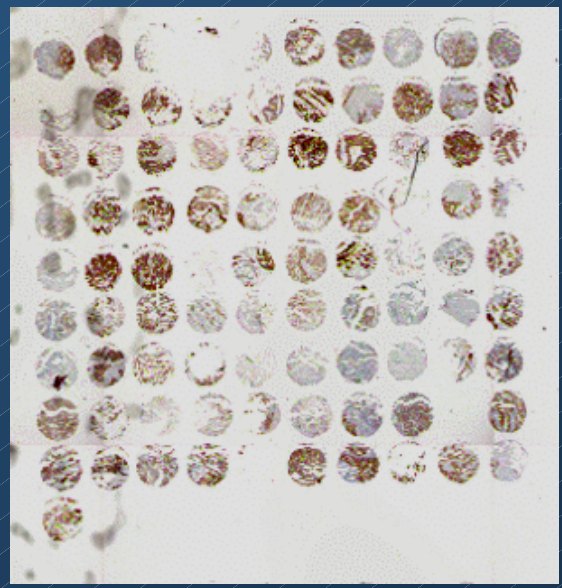
- **High Throughput Pathology**

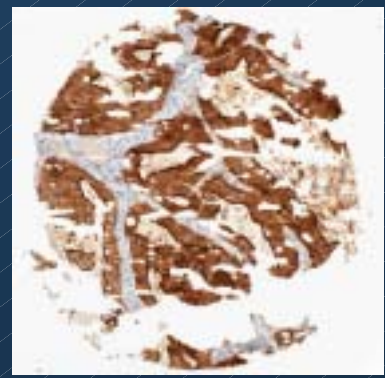
Example: Villin

Differentiate Colonic From Ovarian Adenocarcinoma



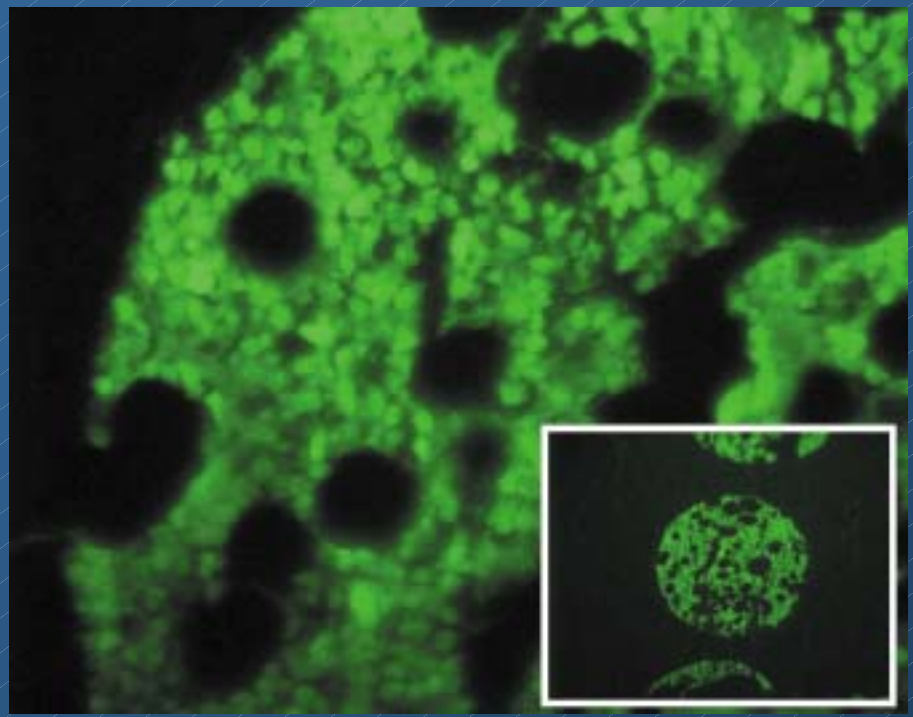




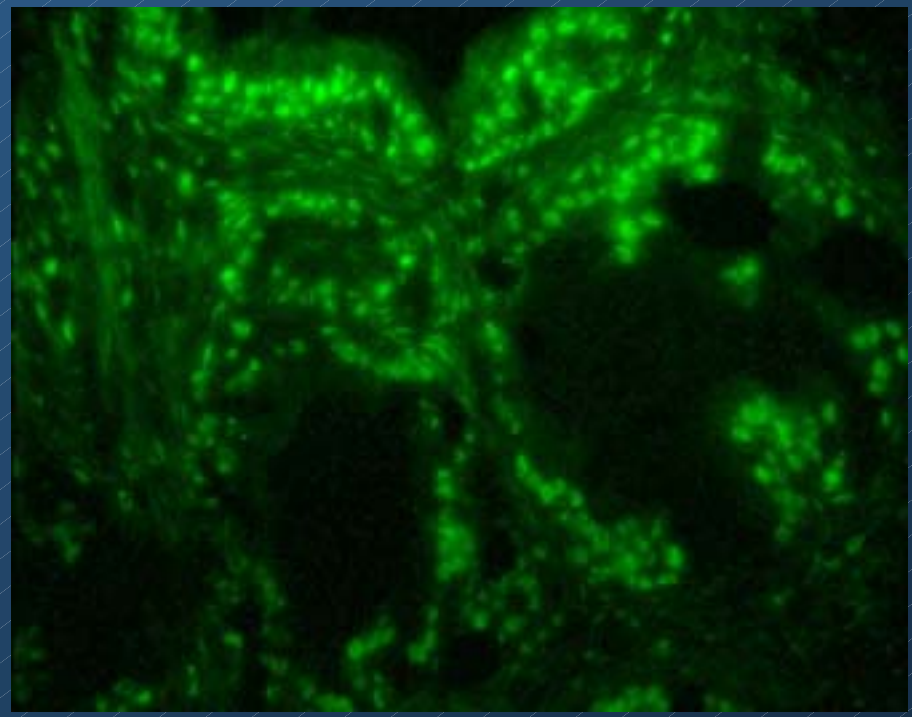


Immunofluorescence

10% Formalin



70% Ethanol



alpha p300

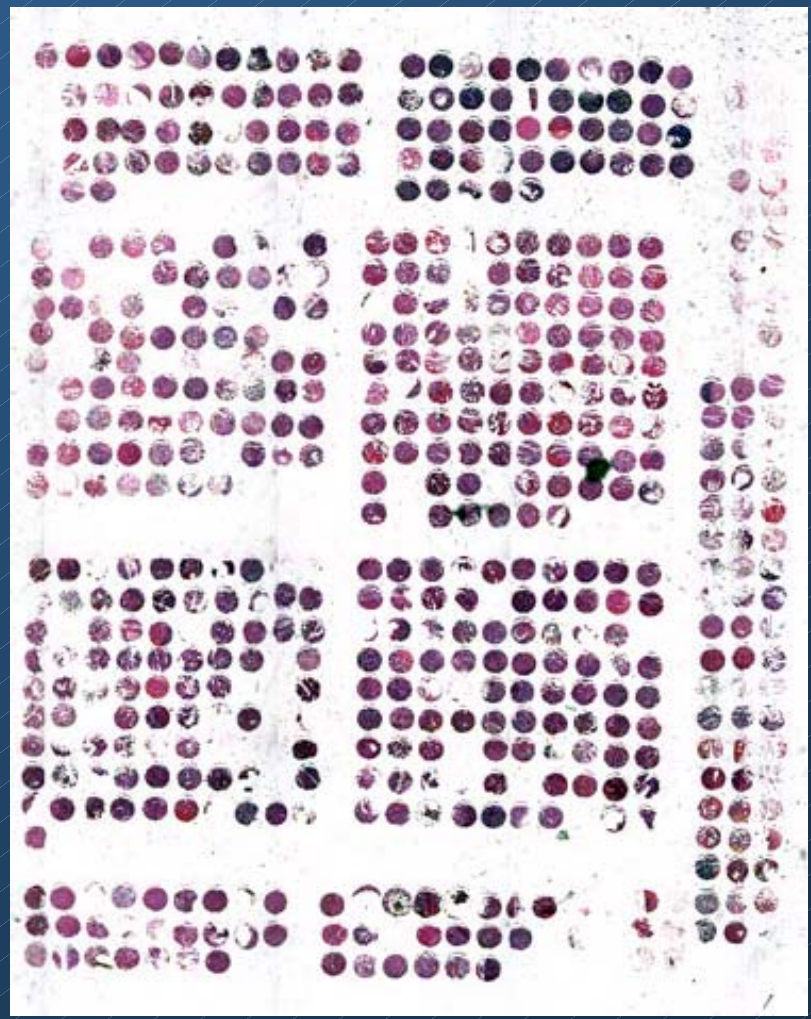
Applications In The Kidney

- Studies Of Renal Tumors
- Studies Of Global Parenchymal Disease
- Studies Of Disease That Differentially Involve The Cortex & Medulla

- Not A Technology Easily Applied To The Glomerulus

Array Construction

1. Design Array
2. Map Donor Slides
3. Build Array Block
4. Section Array Block
5. Stain Array Slides
6. Analyze Data
7. Integrate Data

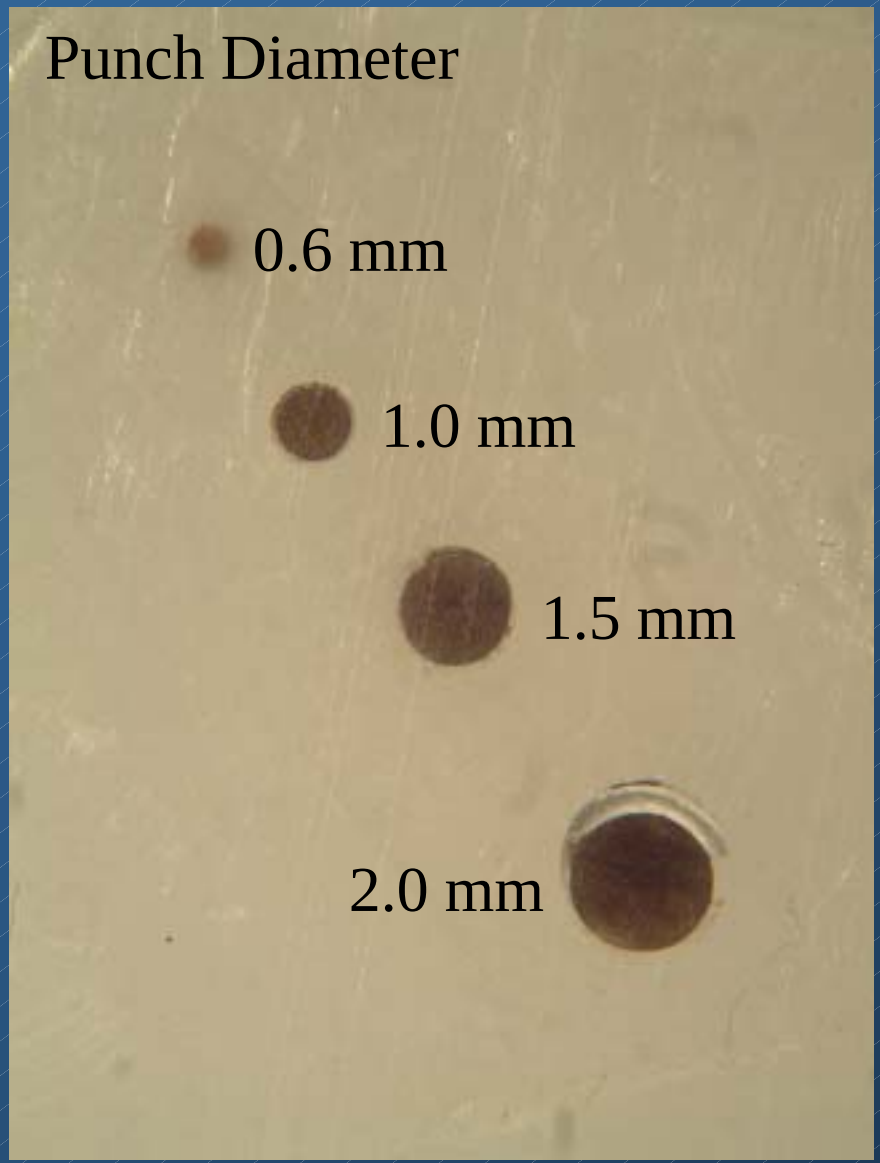


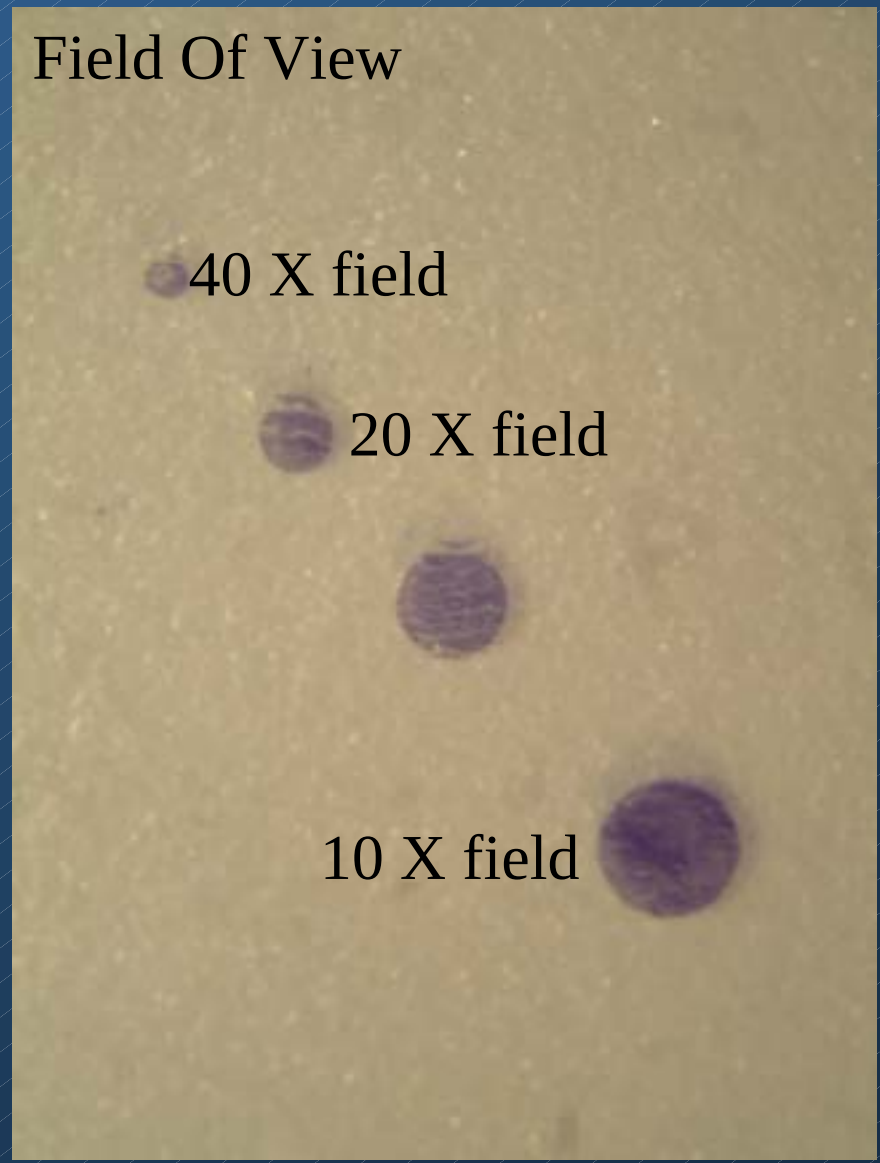
Design

- Density Of Array
  - Spot Size
- Over Sampling
- Normal Tissue
  - Normal Of The Tissue Of Interest
  - Normal From Entire Animal
- Open Space

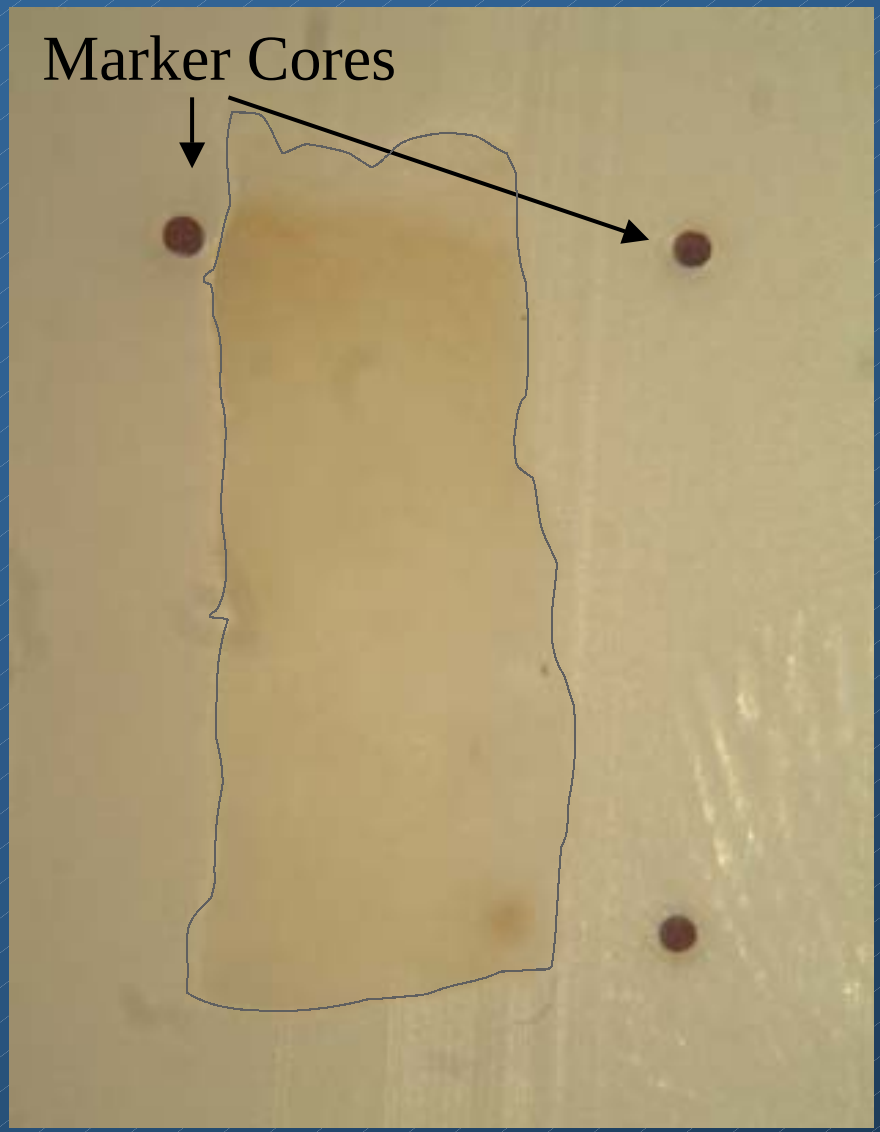
Balancing Act

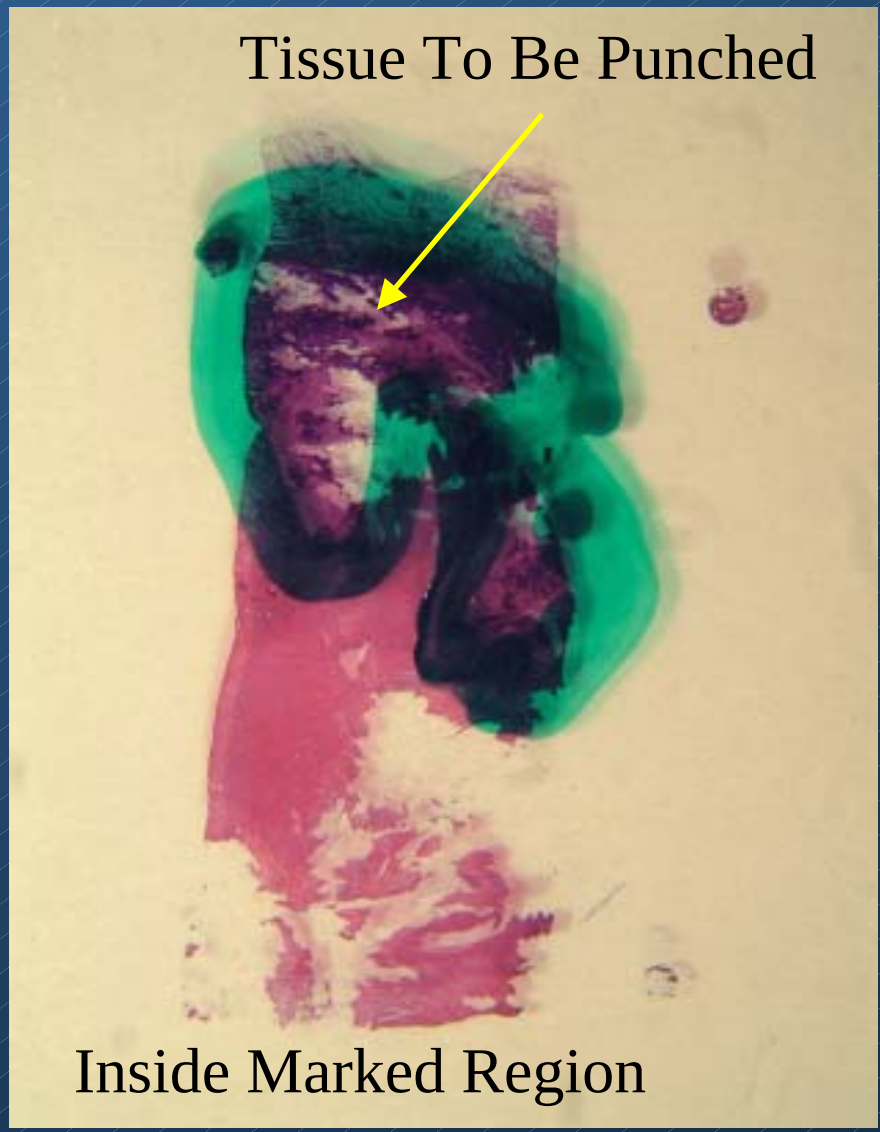
Sample Size Vs. Sample Number



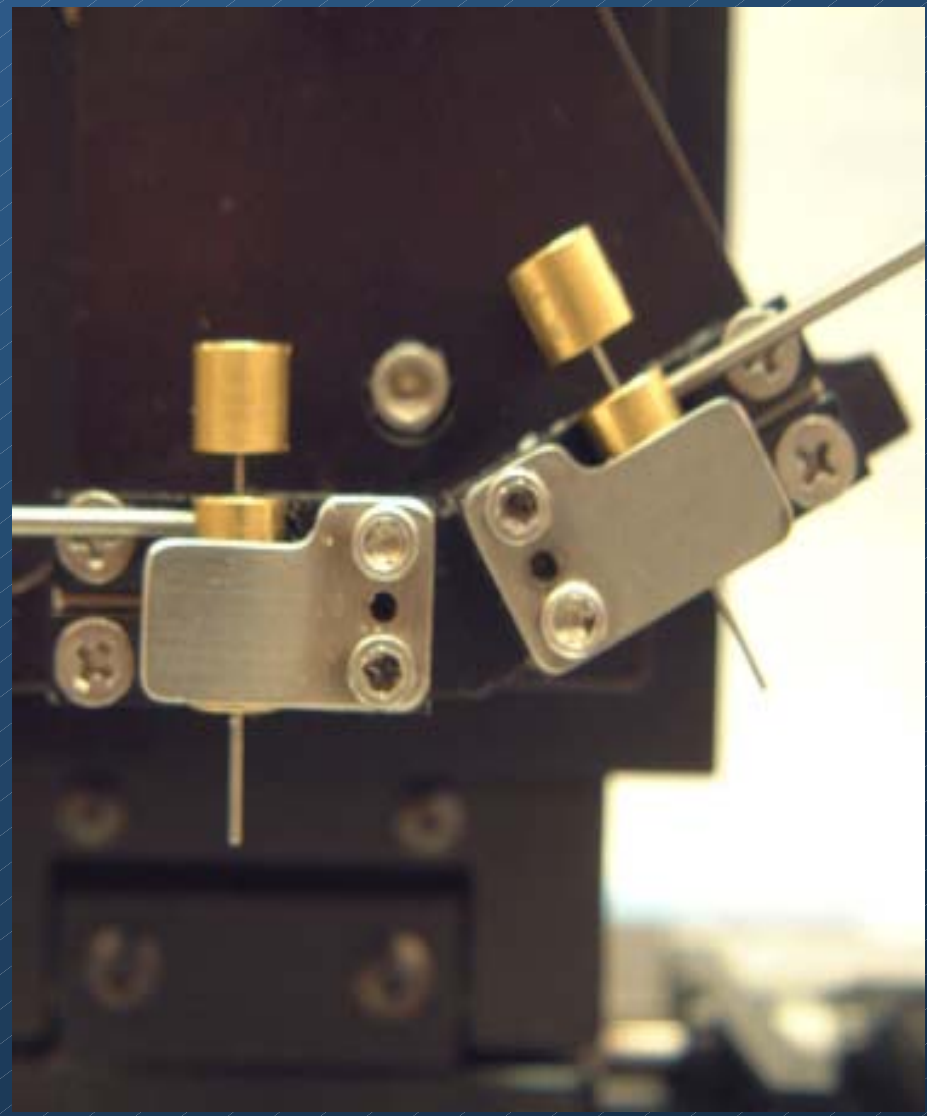


Mapping Donor Tissue



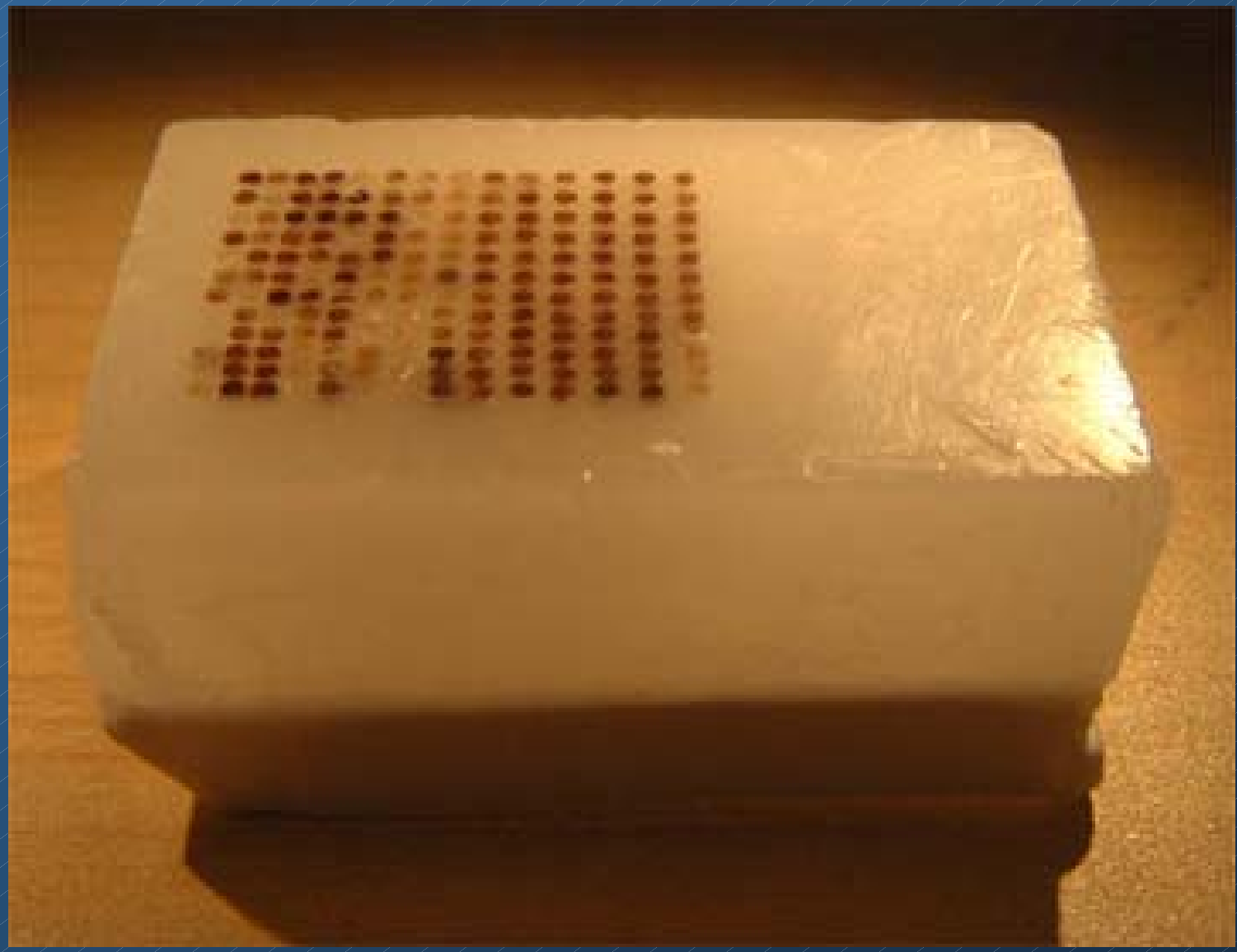


Manual Tissue Arrayer



Block After Donation

An Recipient Array Block



Tape Sectioning

- UV Sensitive Acrylic Coated Slides And Tape Transfer By Instrumedics

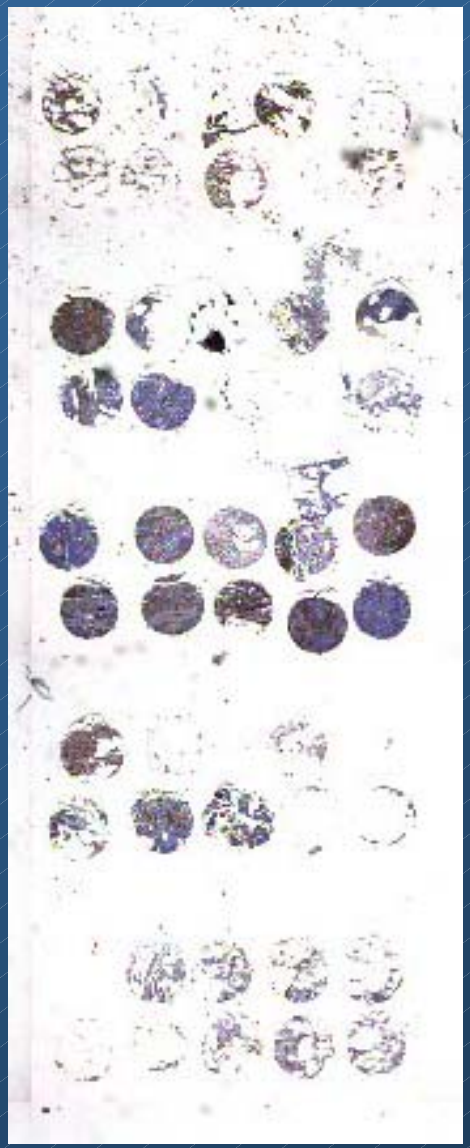
- Benefits
  - No Stretching Or Distortion – Aligned Array
  - No Loss Of Sections – Every Section Used
  - Durable – Withstands All Antigen Retrievals
- Issues
  - Residue – Sticky, Slow To Dehydrate
  - Expensive – Cost Of Slide & Tape
  - Resolution – “Lumpy” Sections

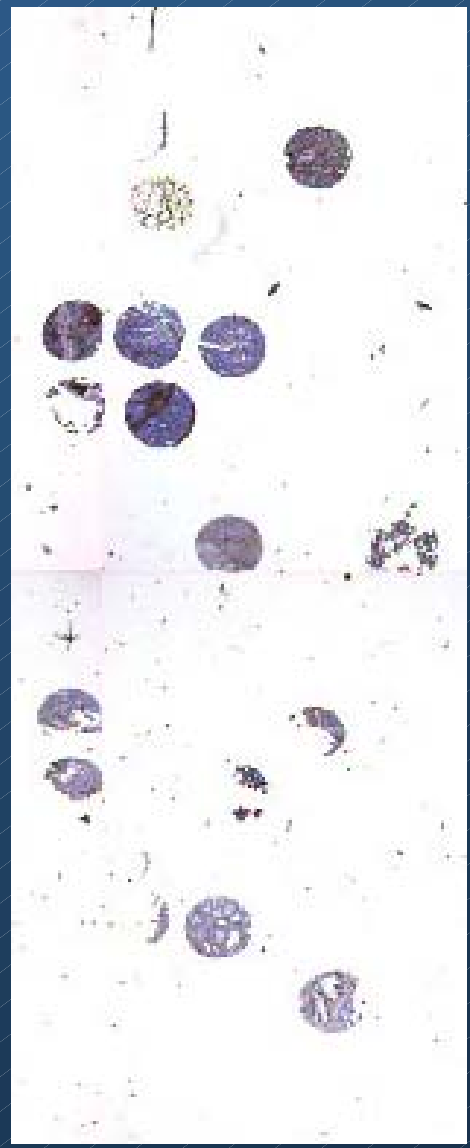
Tape Vs Standard Slides

Tape Section

Improved Retention

No Stretch





Regular Section

Loss Of Spots

Loss Of Alignment

Immunohistochemistry

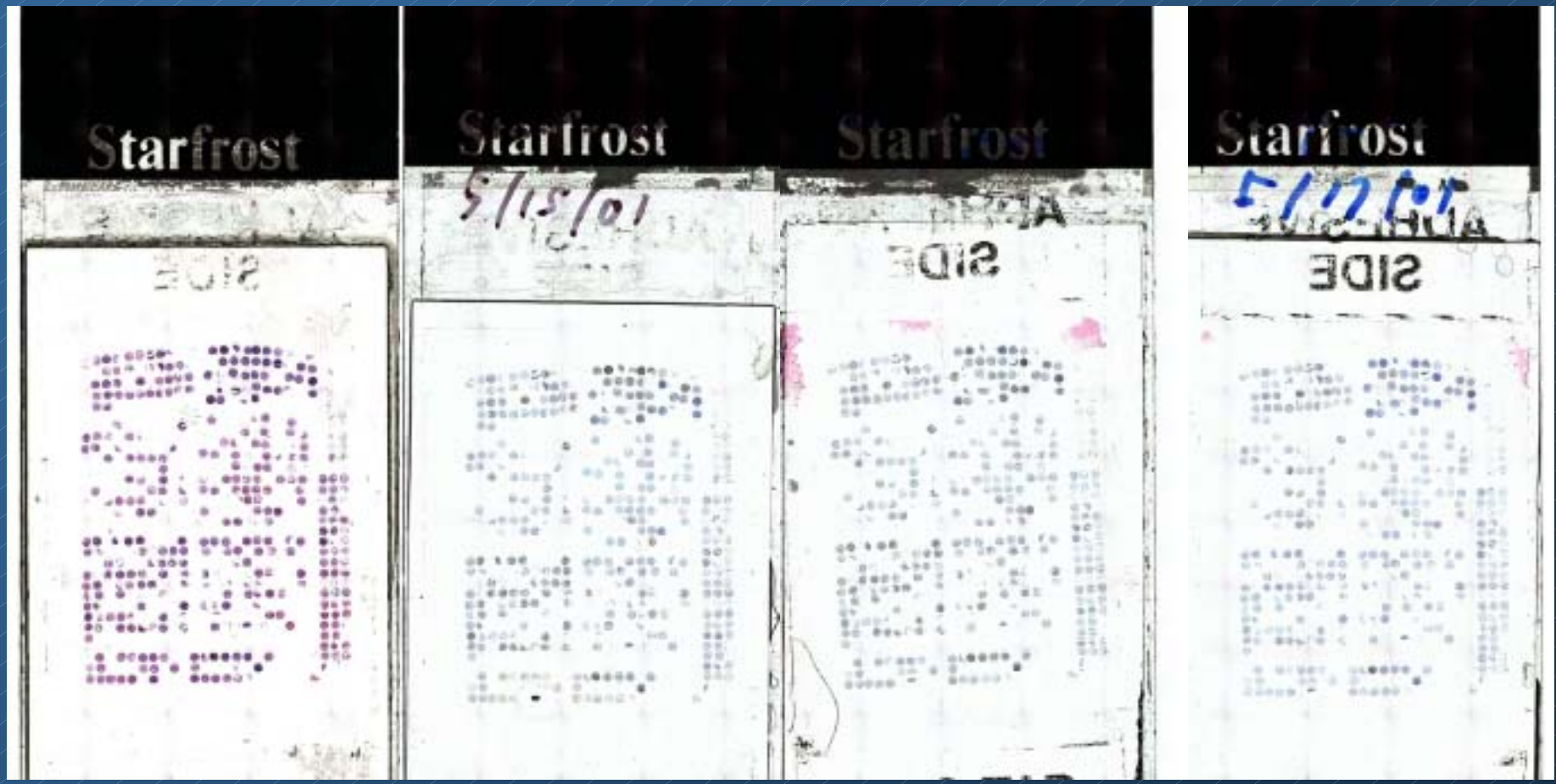
- Antigen Retrieval
  - Very Durable
- Increase Hydration/Dehydration Times 25%
- Increase Incubation Times 25-50%
- Automated Stainers May Pose Problems
- Recommendation
  - Try Surplus Slides First

Now The Real Problem

Collection & Analysis Of The Data

"Overwhelmed By Tissue Arrays! – Pathologist Seeks To Clone Himself"

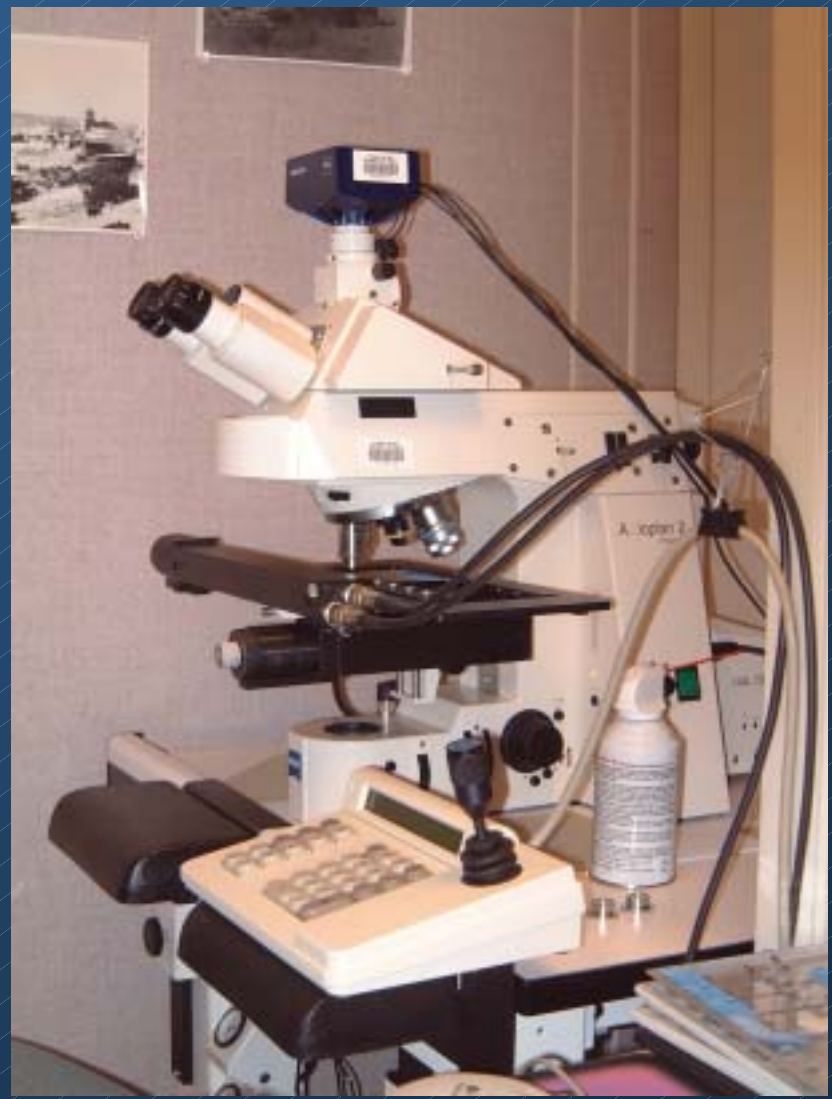
Data Depth



1A151 H&E 1A155 stain1 1A158 stain 2 1A159 stain 3

The Work Horse

The Future Is Here !

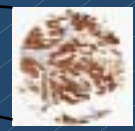


Automated Image Analysis

Montage

Select Region Of Interest

Capture Individual Spots



Interpret / Analyze Spots

Database Results

Interpret Data

Release To
Public Database

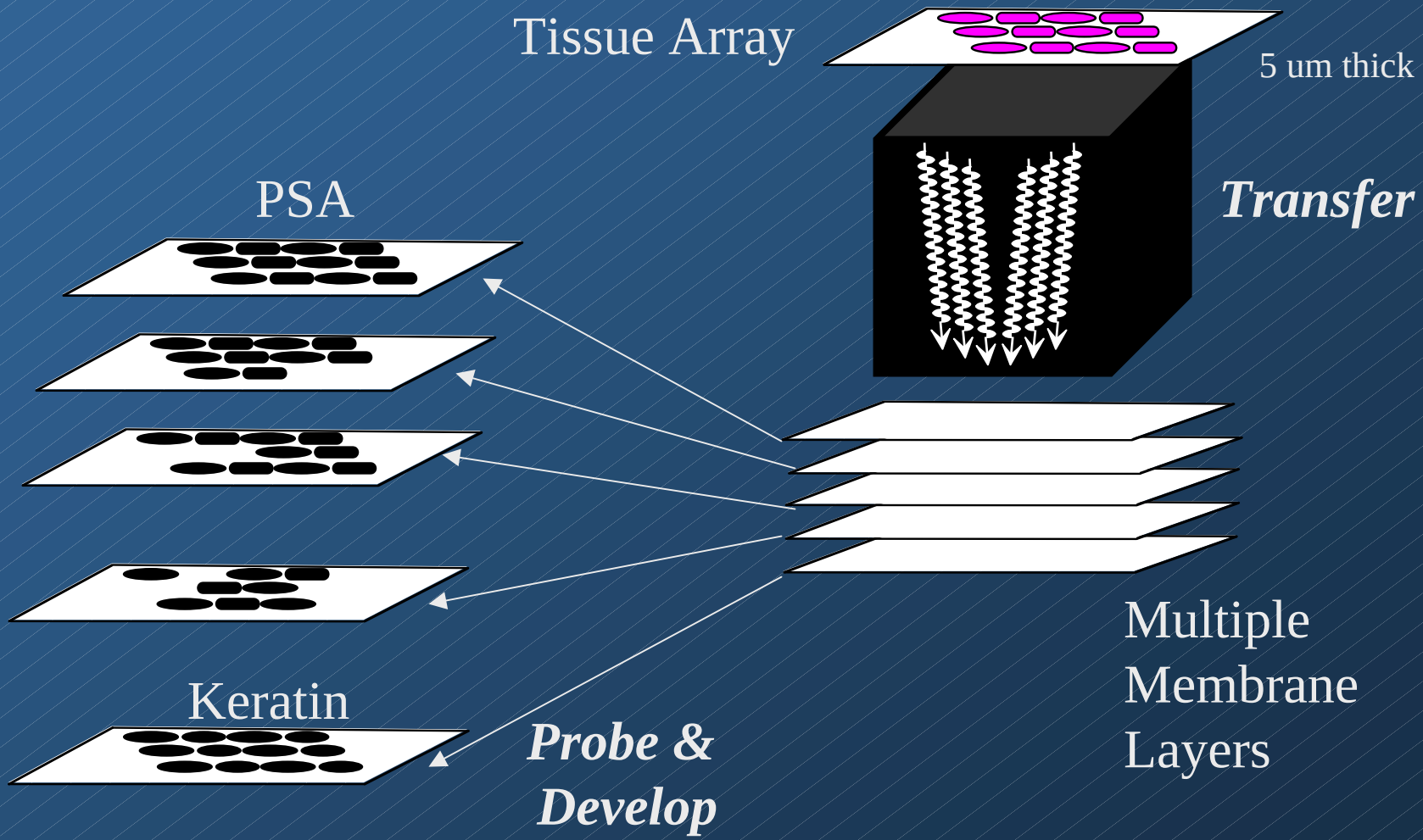
Analyze Additional Stains

Publish

END

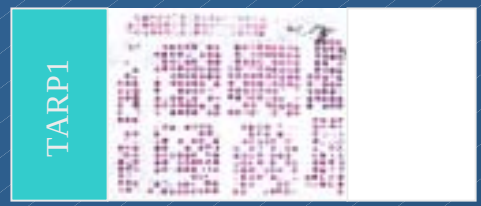
- Limitations Of Tissue Arrays
  - Tissue Changes As One Sections Deeper Into Array
  - Many Antibodies Available Do Not Work In Paraffin
  - Immunohistochemistry Is Not Very Quantifiable
- Goals
  - Multiple Assays On A Single Slide
  - Use A Wide Range Of Antibodies
  - Quantifiable / Internal Reference

Tissue Blotting



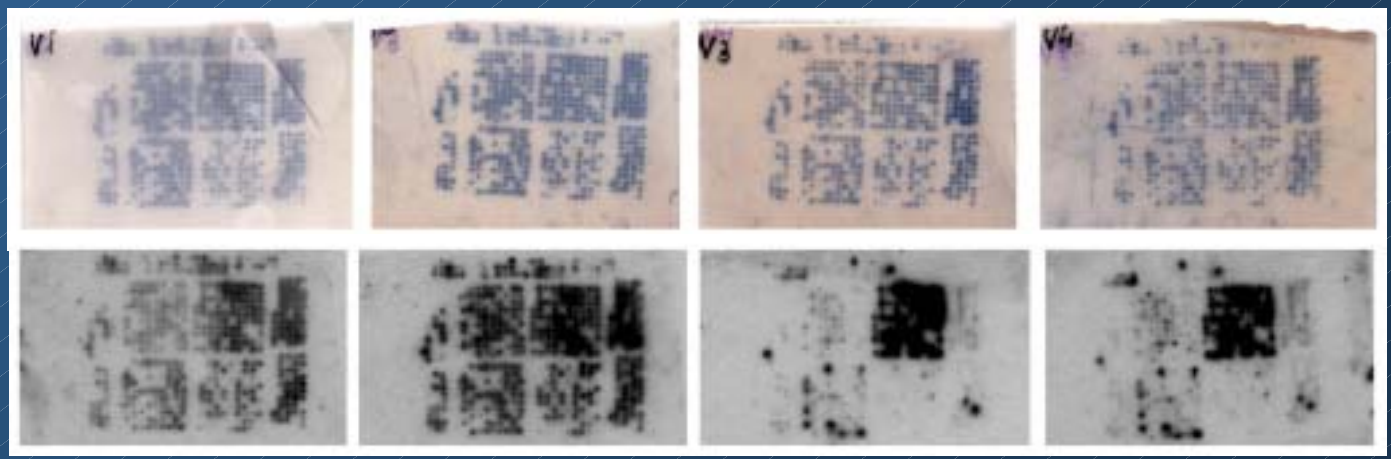
Developed In A CRADA With 20/20 Gene Systems, Inc.

Layered Membrane Analysis Of Tissue Arrays



Total protein

Ab detection



Keratin **Keratin** **PSA** **PSA**

Western Blot Of Transferred Tissue

Proteins eluted in denaturing buffer

1D SDS PAGE

Prostate Tonsil

Transfer Reproducibility

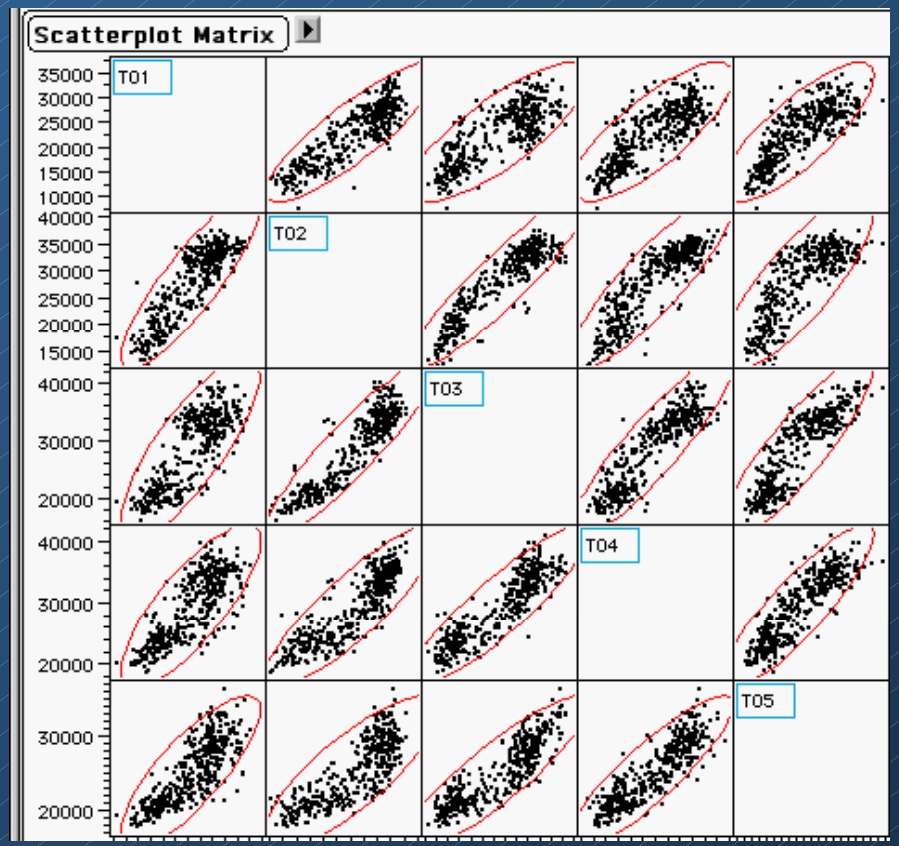
Layer #1

Layer #2

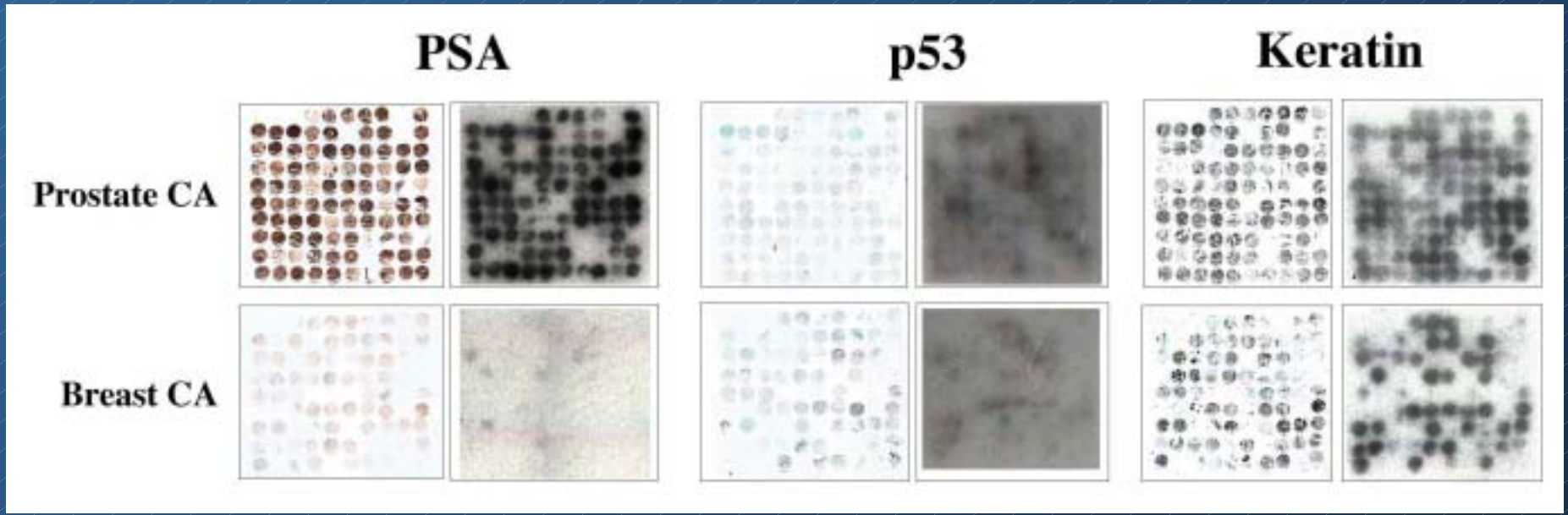
Layer #3

Layer #4

Layer #5



IHC Vs. ECL



Acknowledgements

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Olli Kallioniemi

David Kleiner

Lance Liotta

TARP Lab

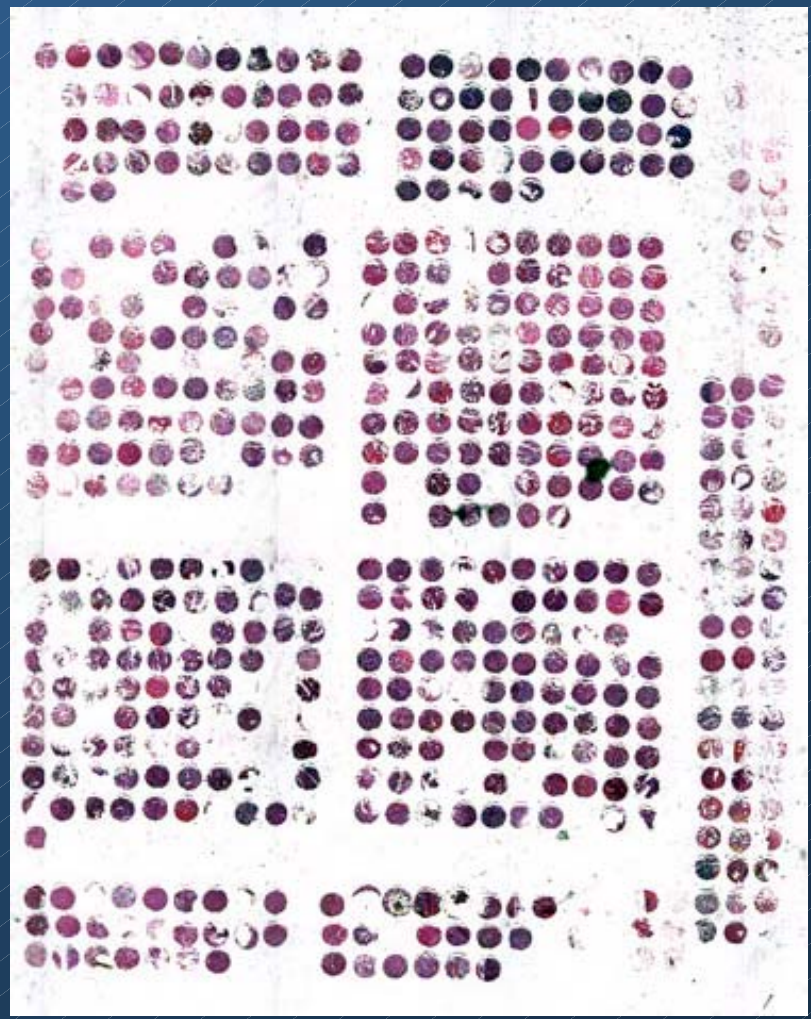
- http://resresources.nci.nih.gov/tarp/
- Genejock@helix.nih.gov
- TARP Lab, ATC, Room 109G, 8717 Grovemont Circle, Gaithersburg, MD 20877
- CHTN, Eastern Division, (215) 662-4570

Is This For Me?

- Equipment Cost
  - Additional Equipment Needed In Addition To A Histology Lab – $10K
  - From Ground Up Cost, Small Scale - $30K
  - Large Scale Full Time Lab - $500K
- Requires A Pathologist & Histotechnologist

Array Construction

1. Designing Array
2. Mapping Donor Slides
3. Arraying
4. Sectioning Of Array Block
5. Staining Of Array Slides
6. Data Analysis

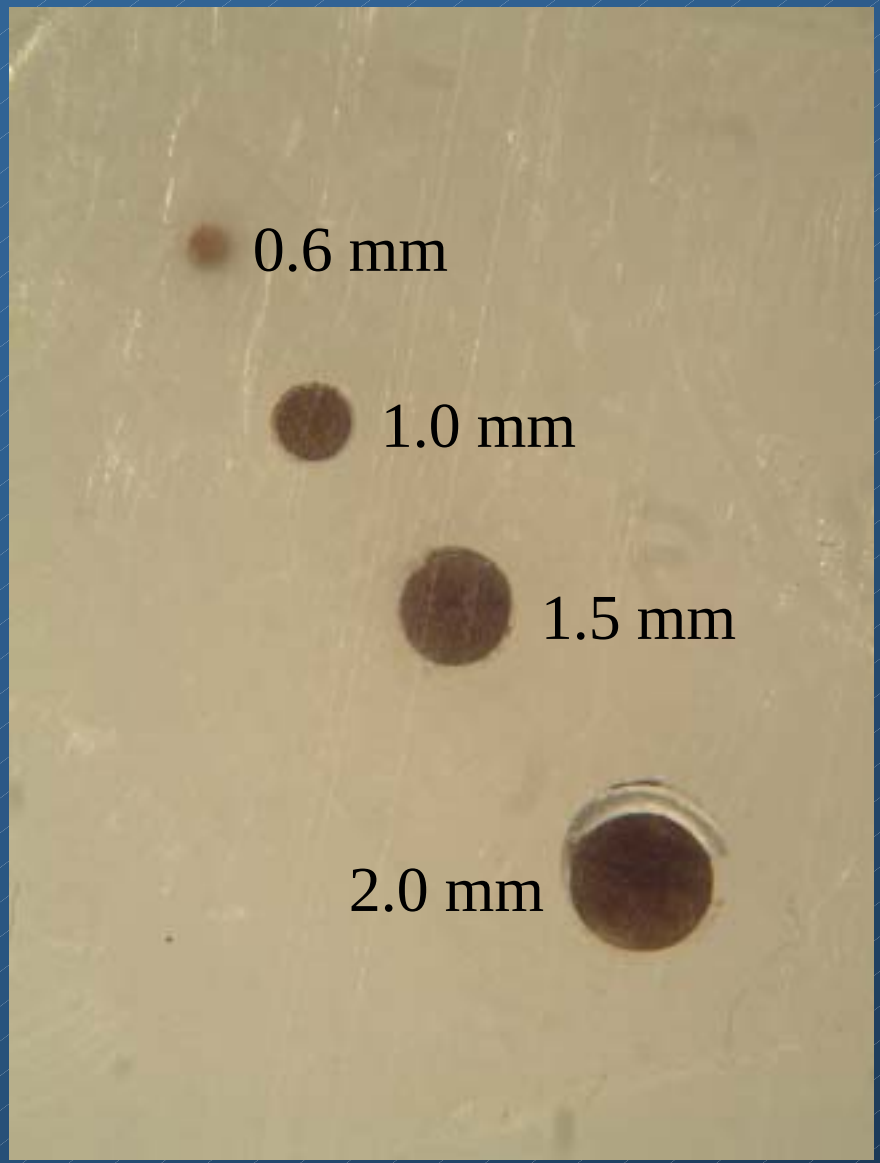


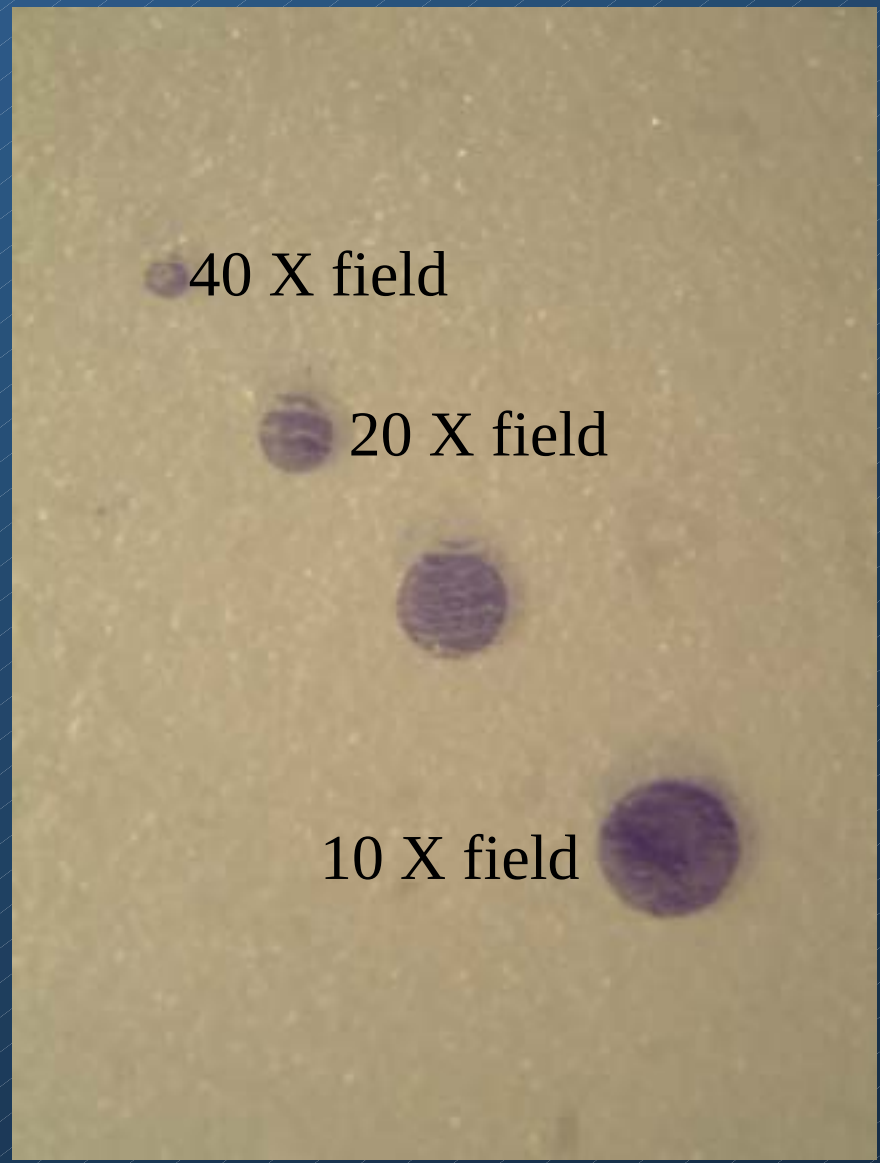
Design Principles

- Over-sampling
- Open Space
- Clusters
- Asymmetry
- Punch Size

Balancing Act

Sample Size Vs. Sample Number



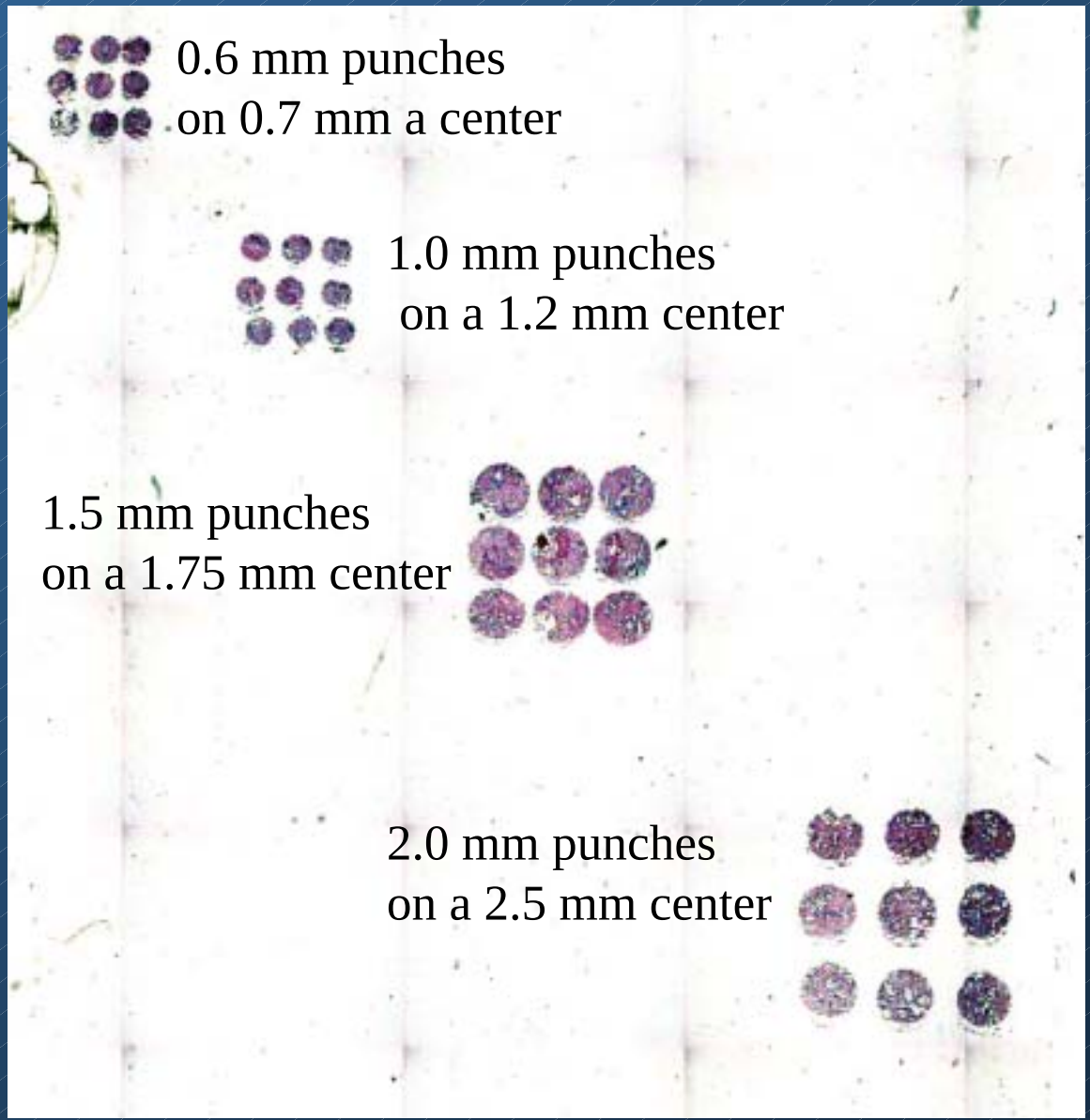


Punch Size

Punch Size	Area	Lens	Array Size
0.6 mm	0.28 mm^2	40x	500
1.0 mm	0.79 mm^2	20x	300
1.5 mm	1.77 mm^2		150
2.0 mm	3.14 mm^2	10x	50

Punch Size

- Punch Size
- Spacing



Design

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- Normal Tissue
  - Normal Of The Tissue Of Interest
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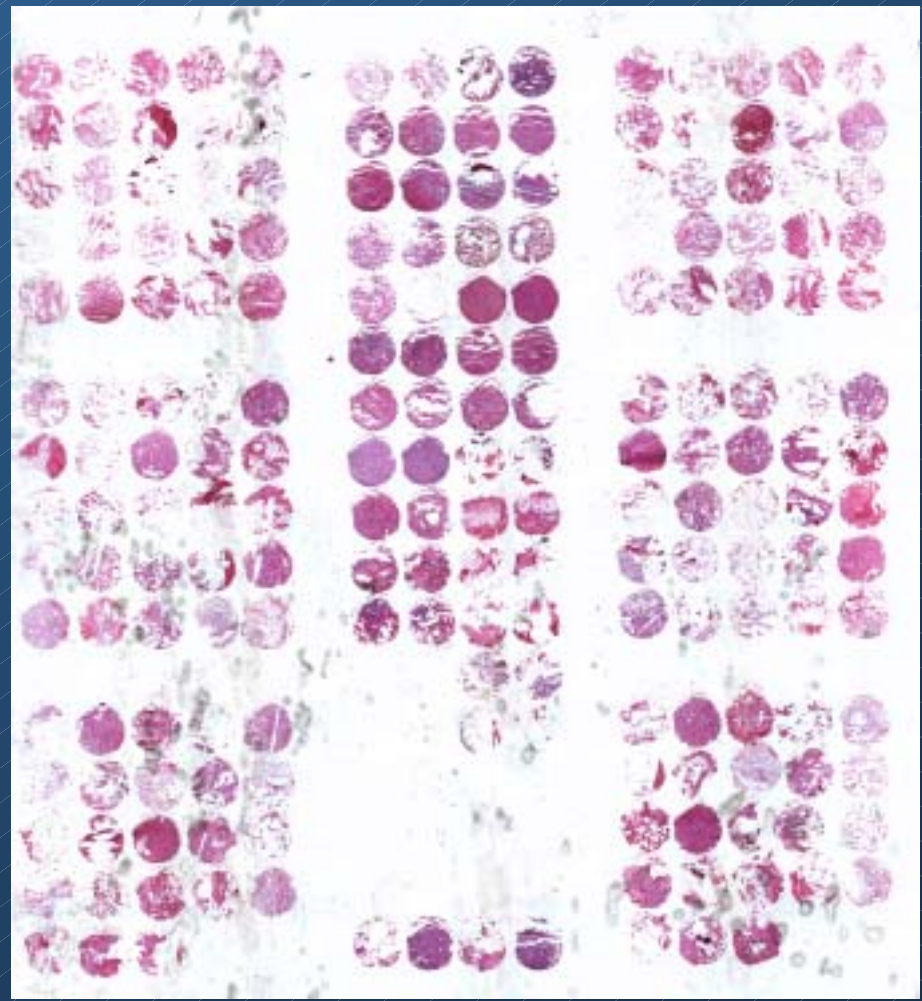
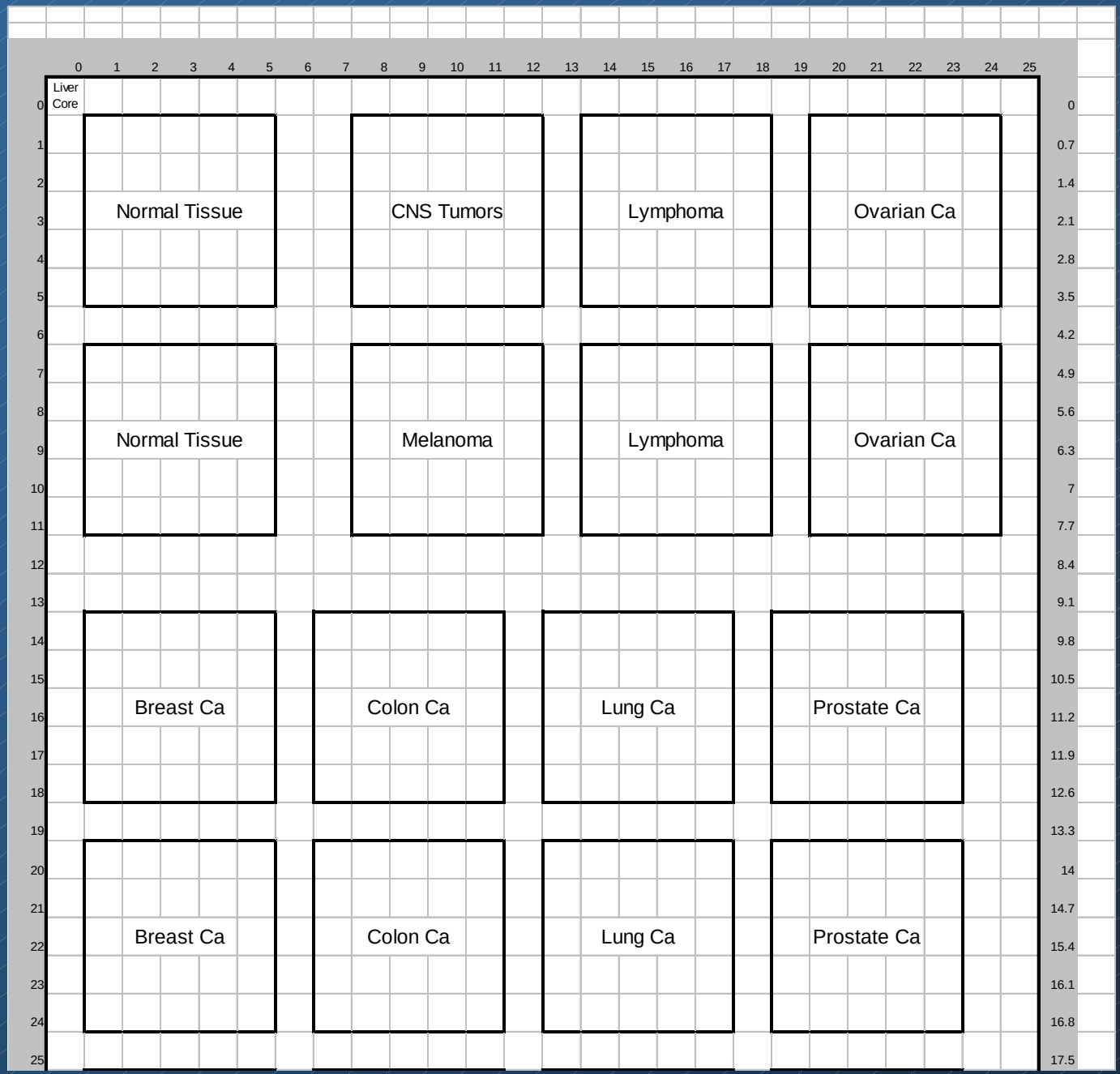
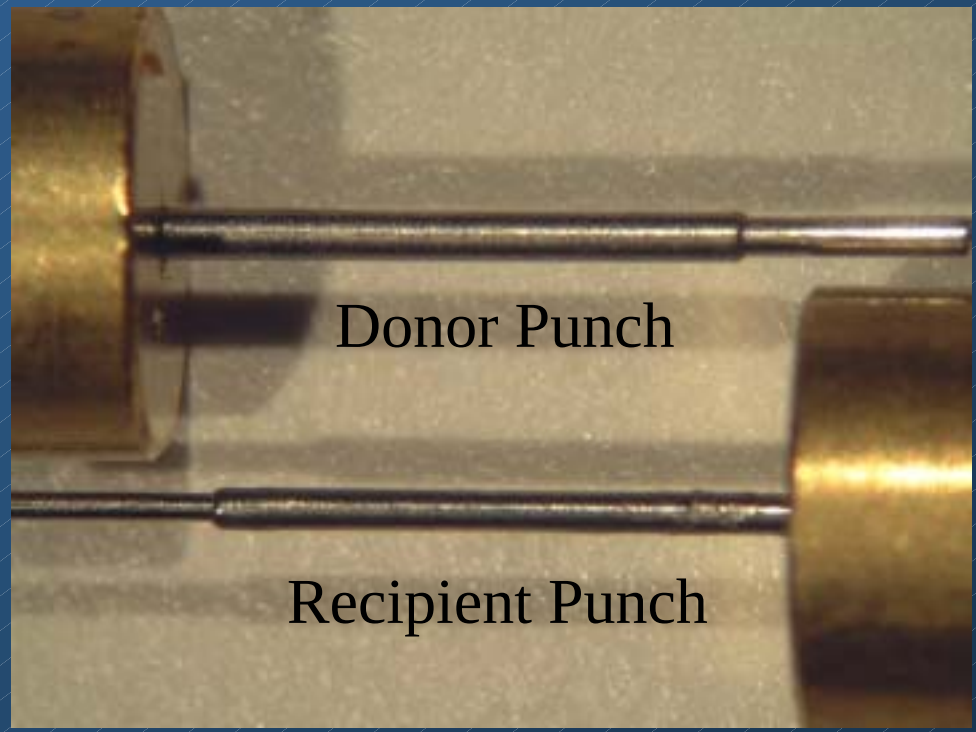


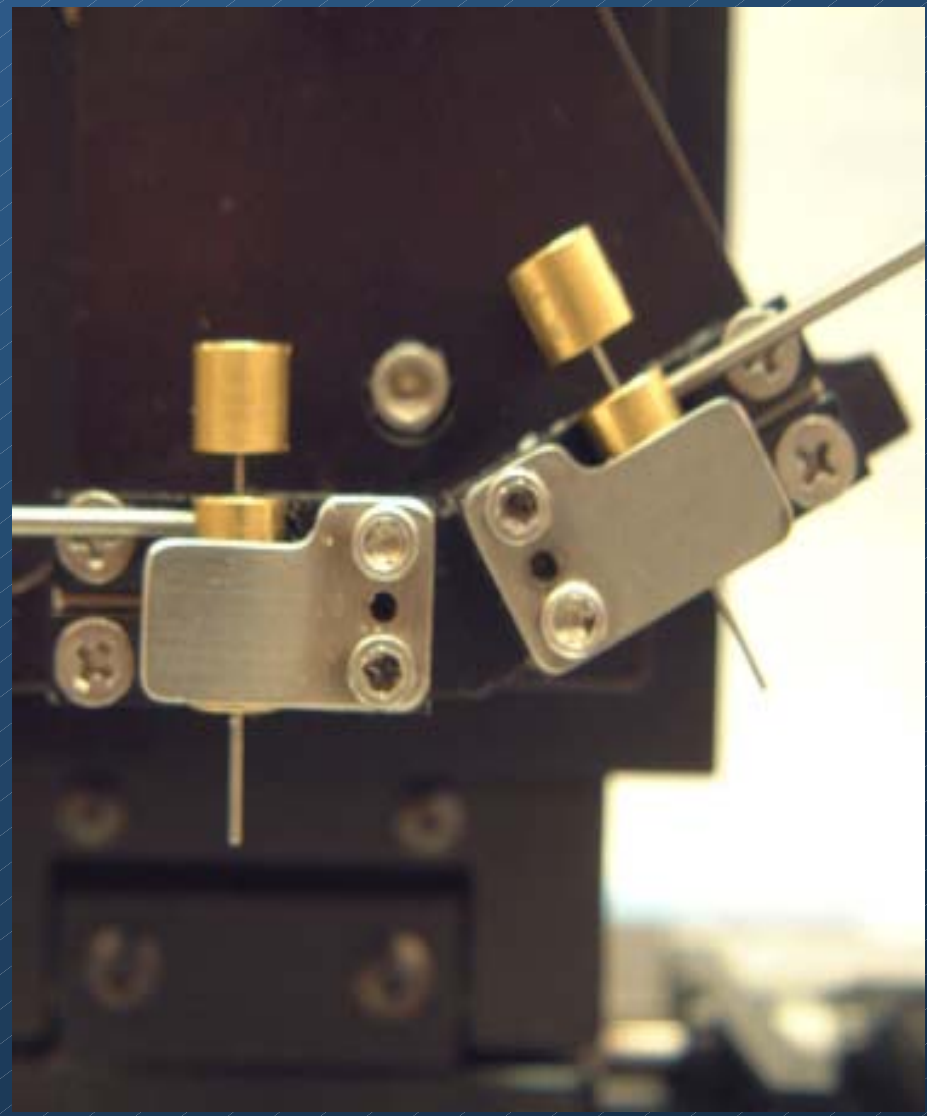

Liver Core
Normal Tissue
CNS Tumors
Lymphoma
Ovarian Ca
Normal Tissue
Melanoma
Lymphoma
Ovarian Ca
Breast Ca
Colon Ca
Lung Ca
Prostate Ca
Breast Ca
Colon Ca
Lung Ca
Prostate Ca

Arrayer Needles

- Recipient Punch External Diameter Equals The Internal Diameter Of The Donor Punch



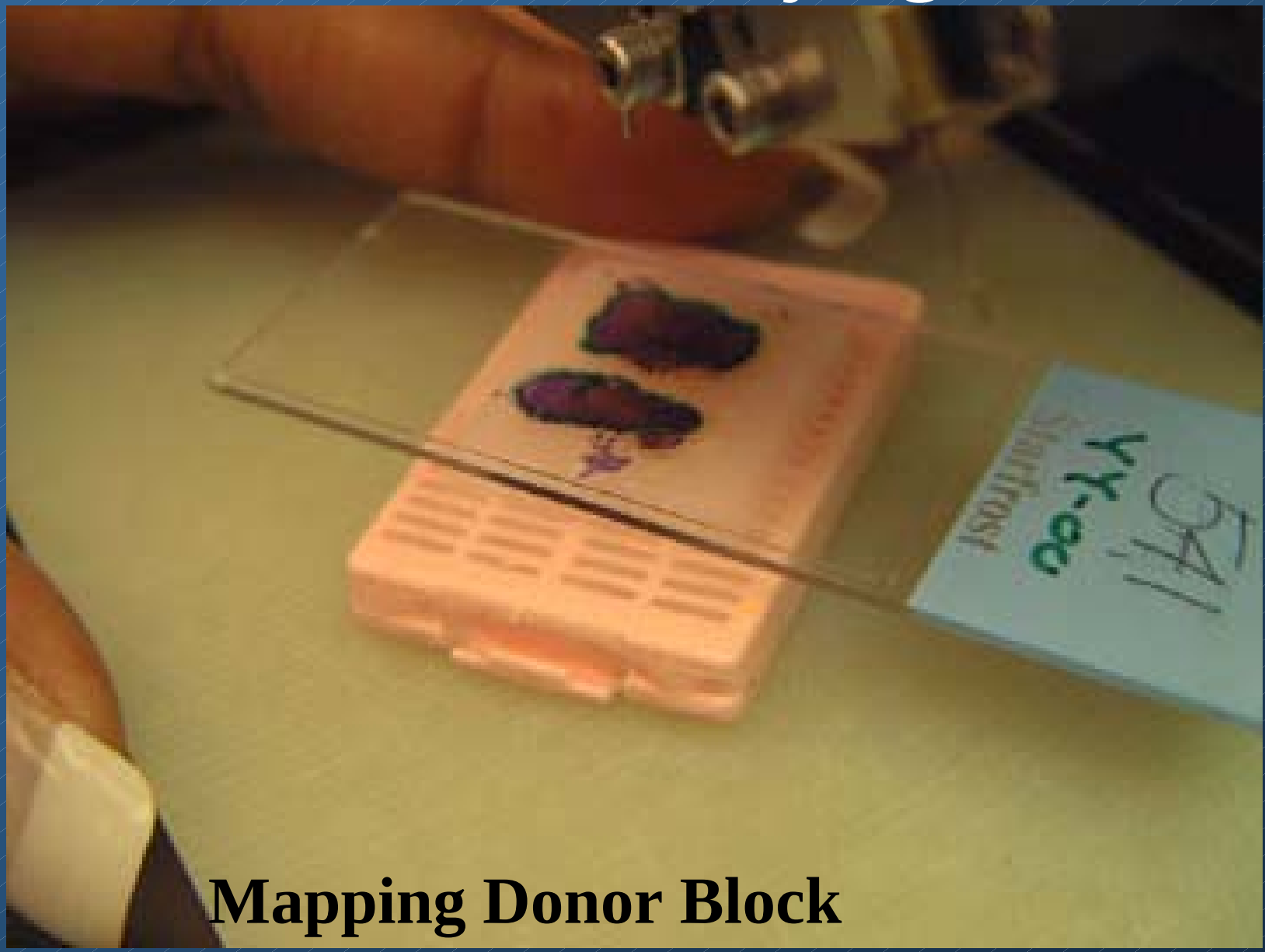
Manual Tissue Arrayer



Manual Arraying

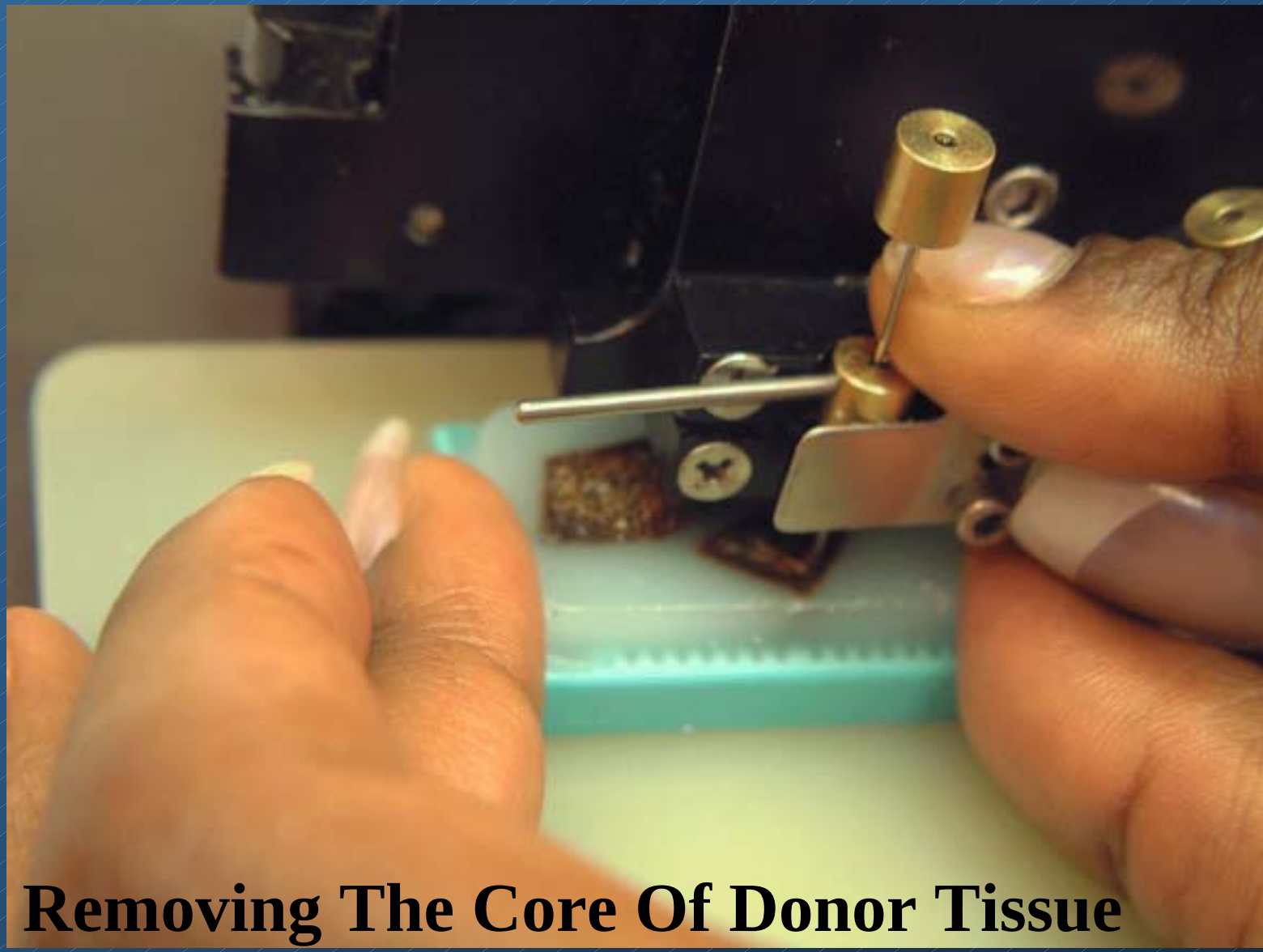
Making A Hole In The Recipient Block

Manual Arraying



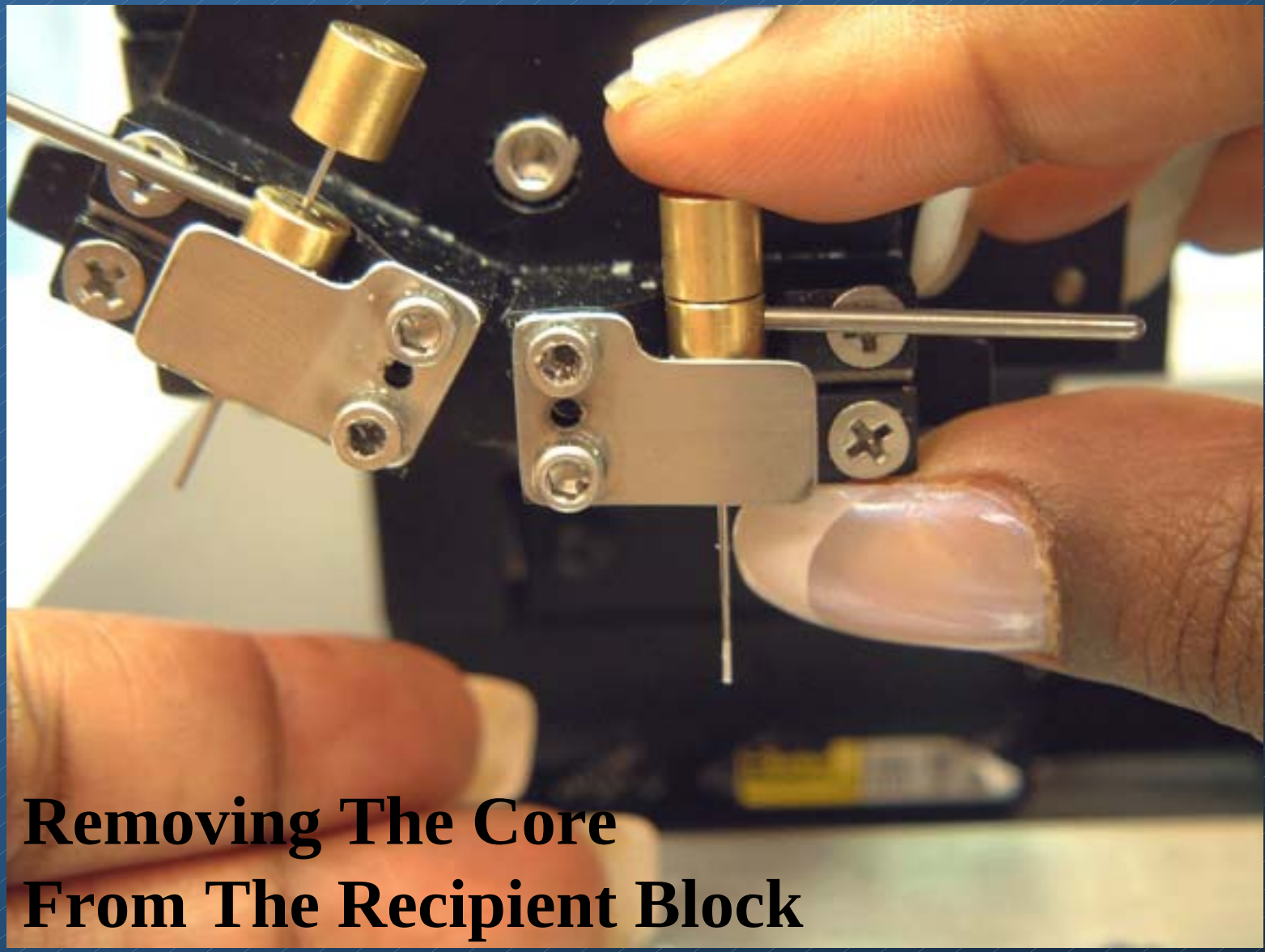
Mapping Donor Block

Manual Arraying



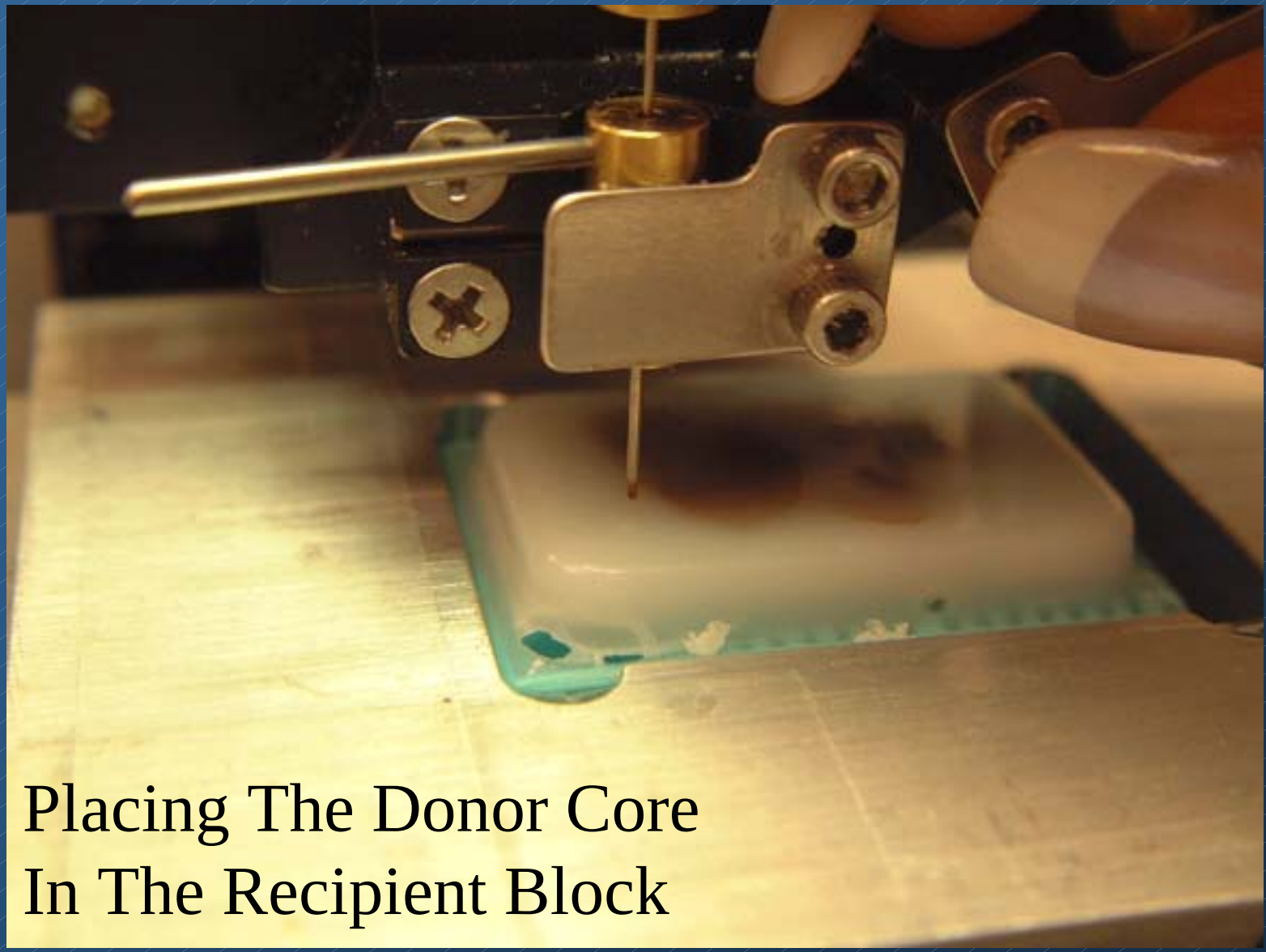
Removing The Core Of Donor Tissue

Manual Arraying



Removing The Core
From The Recipient Block

Manual Arraying



Placing The Donor Core
In The Recipient Block

Block After Donation

Tape Sectioning

- UV Sensitive Acrylic Coated Slide and Tape Transfer By Instrumedics

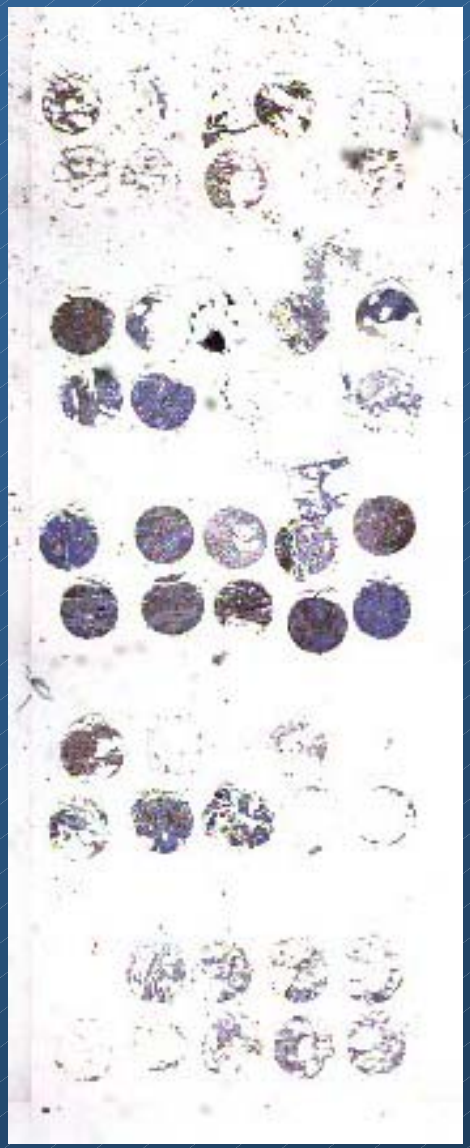
- Benefits
  - No Stretching Or Distortion – Aligned Array
  - No Loss Of Sections – Every Section Used
  - Durable – Withstands All Antigen Retrievals
- Issues
  - Residue – Sticky, Slow To Dehydrate
  - Expensive – Cost Of Slide & Tape
  - Resolution – “Lumpy” Sections

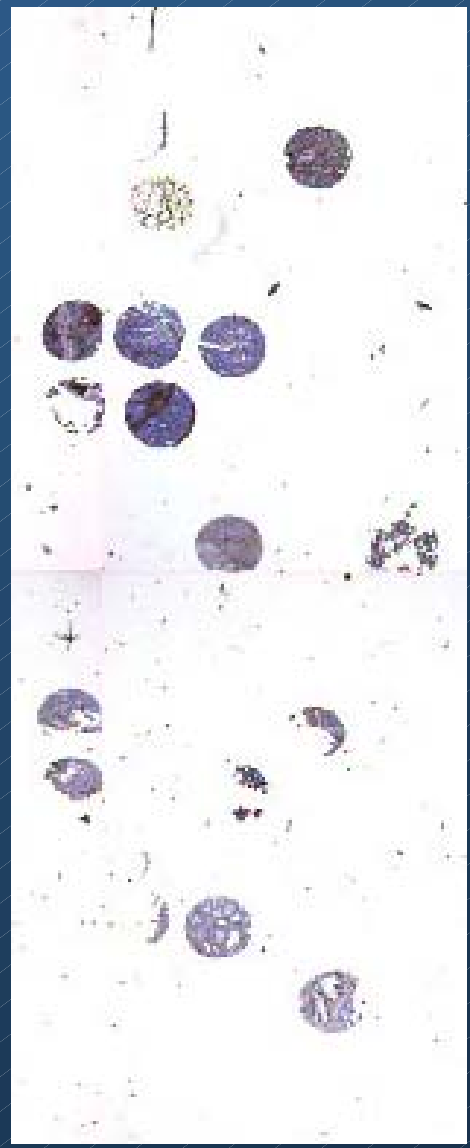
Tape Vs Standard Slides

Tape Section

Improved Retention

No Stretch





Regular Section

Loss Of Spots

Loss Of Alignment

Immunohistochemistry

- Antigen Retrieval
  - Very Durable
  - Some Problems With High pH For Long Durations
- Increase Hydration/Dehydration Times 25%
- Increase Incubation Times 25-50%
- Automated Stainers May Pose Problems
- Recommendation
  - Try Surplus Slides First

Issues Concerning Human Tissue

- Patient Material Requires IRB Approval
- Archival Material
  - Formalin Fixed
  - Variable Fixation and Processing Conditions
- Prior Use Of Blocks Decreases The Number Of Usable Sections Obtainable

Animal Tissue – Plan Ahead

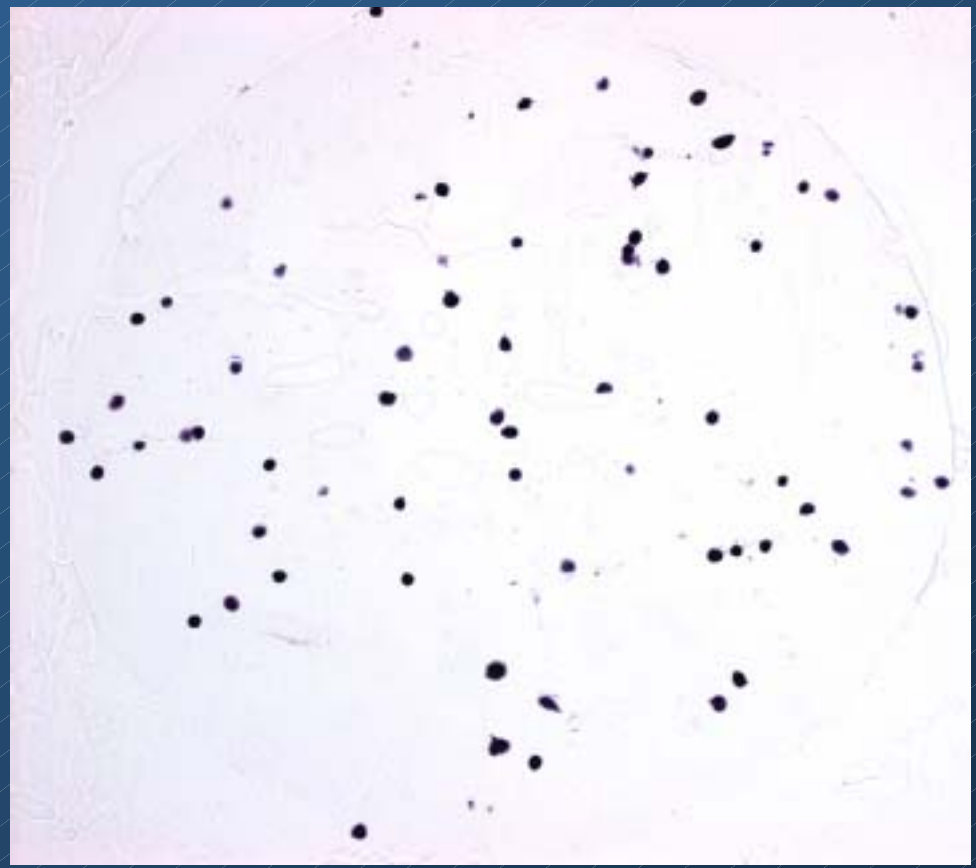
- Fixatives
  - Formalin
    - Decalcification With EDTA
  - 70% Ethanol
- Controlled Processing
- Customized Blocking & Preparation For Arraying

70 % Ethanol As A Fixative

- Improved Recovery Of Nucleic Acids
- No Cross-linking Of Proteins
- No Auto-Fluorescence
- Conditions For Immunohistochemistry Resemble Those Of Frozen Tissue
- Minimal Change In Histology
- Only Change Is Removal Of First Step On Processor

Cell Block Construction

- Embed Cells In Agarose
- Process As Tissue



Resources

- Arrayers: Beecher Instruments-
  - http://www.beecherinstruments.com/
- Tape Sectioning: Instrumedics-
  - http://www.instrumedics.com/
- NCI TARP LAB
  - http://resresources.nci.nih.gov/tarp/index.cfm
- NHGRI Tissue Array Lab
  - http://www.nhgri.nih.gov/DIR/CGB/TMA/
- Layered Membranes - 20/20 Gene Systems, Inc.
  - http://www.2020gene.com/

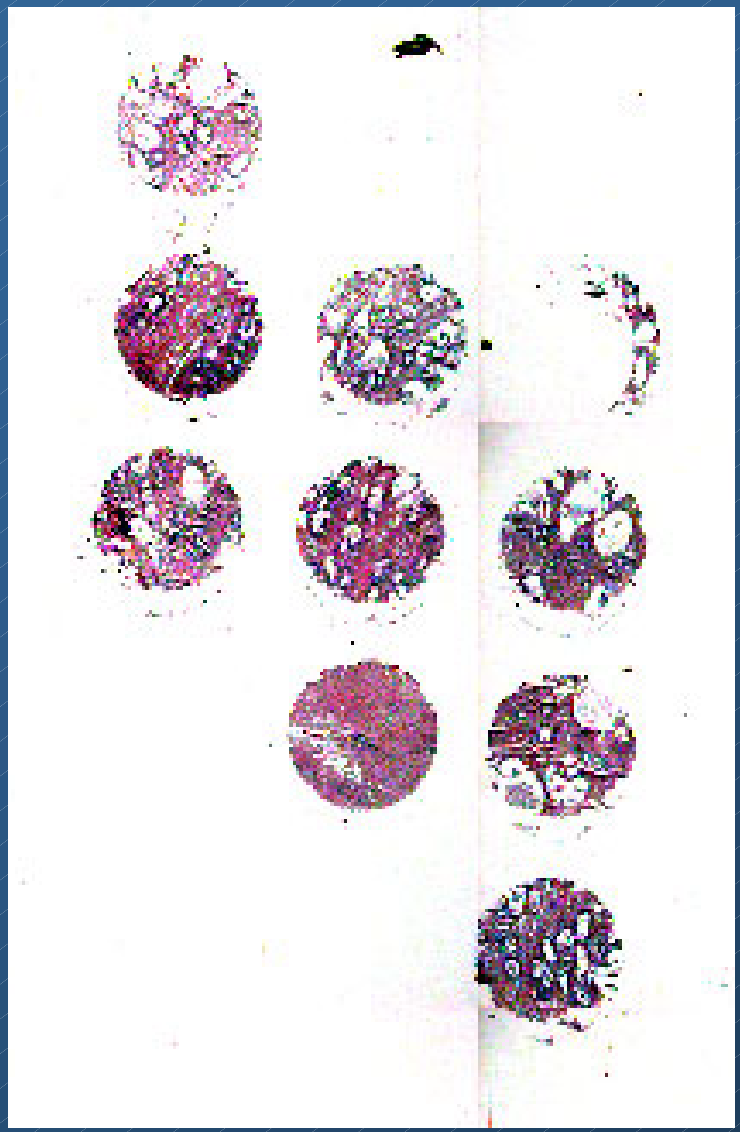
TARP Lab

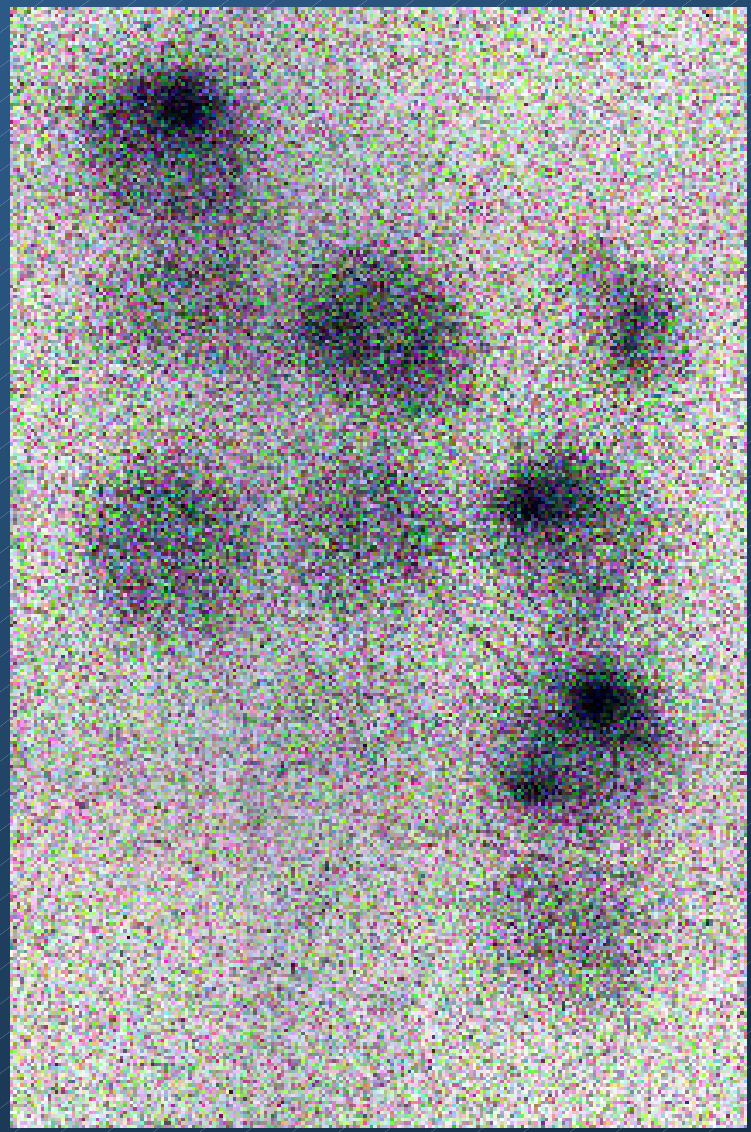
- http://resresources.nci.nih.gov/tarp/
- Genejock@helix.nih.gov
- TARP Lab, ATC, Room 109G, 8717 Grovemont Circle, Gaithersburg, MD 20877
- CHTN, Eastern Division, (215) 662-4570

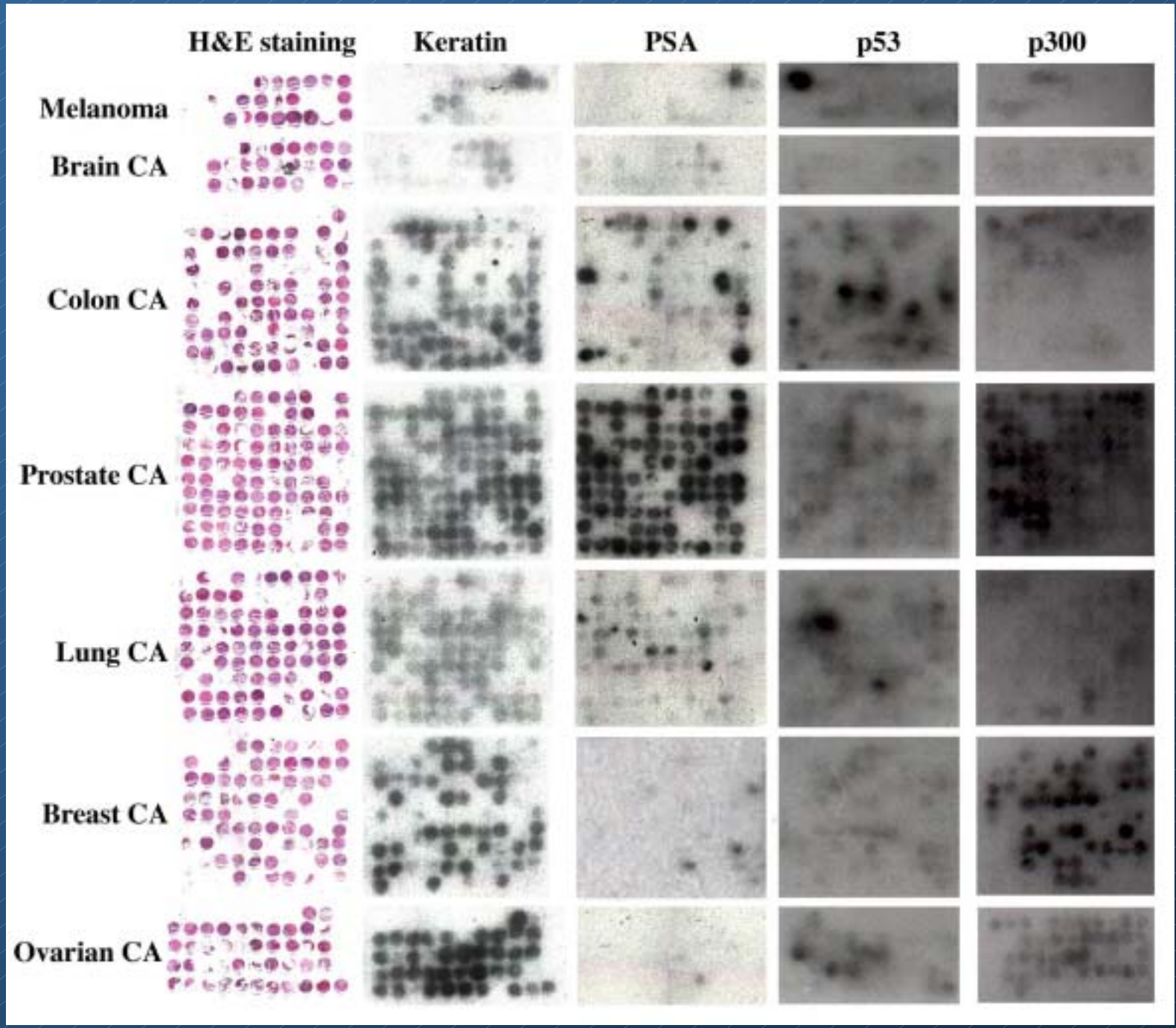
If You Use TARP Slides:

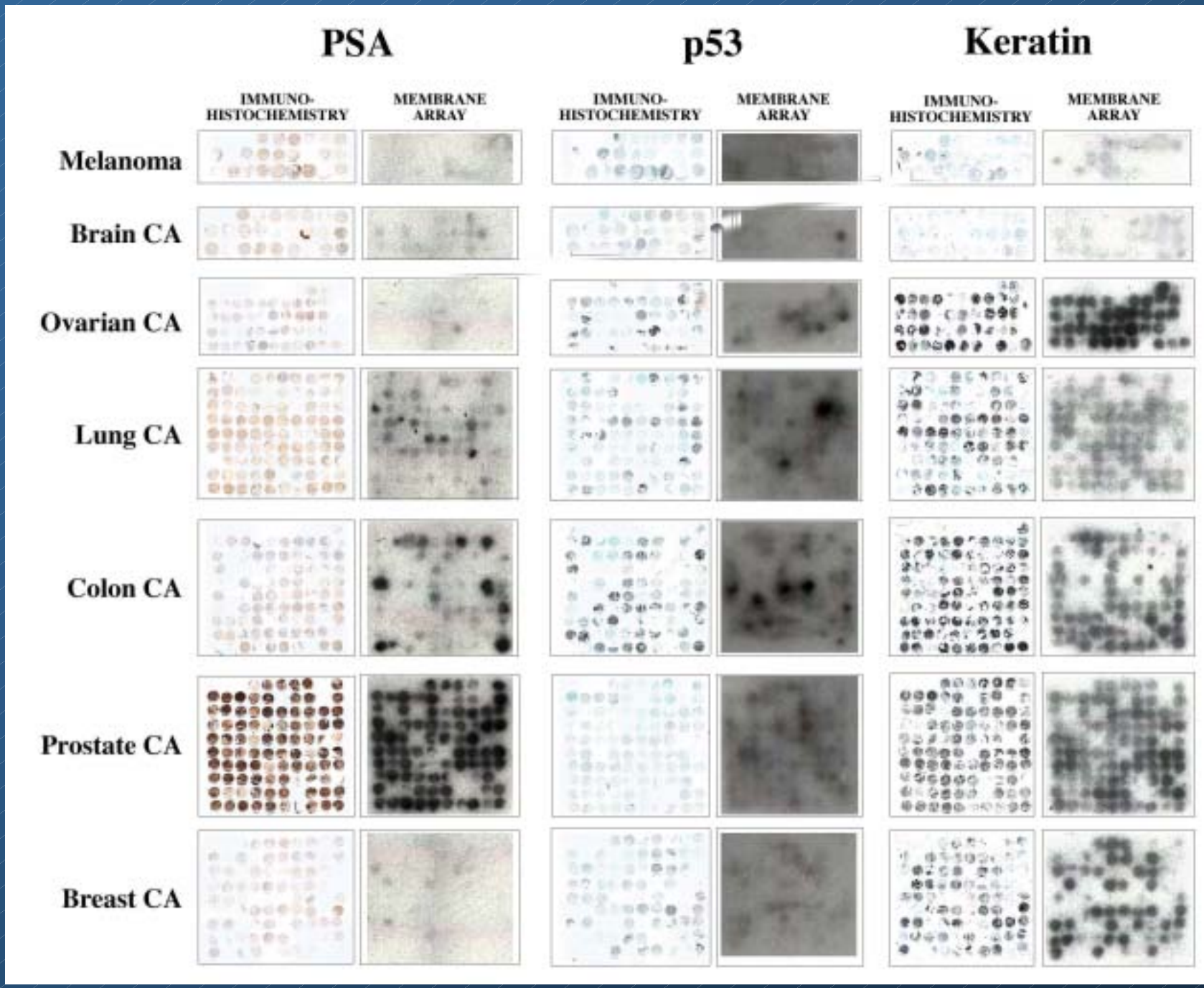
- Multiple-tumor tissue micro-array slides were obtained from the Tissue Array Research Program (TARP) of The National Cancer Institute, The National Institutes of Health, Bethesda, MD 20892.

Resolution Of Transfer






H&E staining
Keratin
PSA
p53
p300
Melanoma
Brain CA
Colon CA
Prostate CA
Lung CA
Breast CA
Ovarian CA


PSA
p53
Keratin
IMMUNO-HISTOCHEMISTRY
MEMBRANE ARRAY
IMMUNO-HISTOCHEMISTRY
MEMBRANE ARRAY
IMMUNO-HISTOCHEMISTRY
MEMBRANE ARRAY
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Mark Raffeld
Steve Chen
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